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BLOOD AND URINE CHEMISTRY

PLATE I.—STANDARD WEDGES.

1. Standard Phenolsulphonphthalein Wedge.
2. Standard Uric Acid Wedge.
3. Standard Nitrogen Wedge.
4. Standard Cholesterol Wedge.

THE NEWER METHODS OF BLOOD AND URINE CHEMISTRY

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TO
WILLIAM MARION REEDY
AN ESTEEMED FRIEND

PREFACE

The present work was undertaken in response to a demand from our many professional friends who have become keenly interested in this line of laboratory investigation. We lay but little claim to originality but feel that if we have collected the major part of the information that is so widely scattered throughout the journal literature of the past three or four years, and boiled it down into a readily digested form, our labors will not have been in vain. The investigations in blood chemistry are proceeding rapidly so that, of necessity, this sort of book will be difficult to keep up-to-date. We, therefore, ask for the indulgence of those who are insistent upon the very last word.

It will be noted that, in the main, we have given but one method for each test. We have done this, because we believe we know what the majority of the practical workers along this line judge the best test to be: besides, we see no reason for describing tests that time and experience have proved fallacious or too complicated. The work in hand gives the technic just as we carry out our routine and research work in our laboratories.

R. B. H. G.

A. J. B.

St. Louis, Mo.

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BLOOD AND URINE CHEMISTRY

PART I.

TECHNIC OF BLOOD CHEMISTRY

CHAPTER I.

GENERAL CONSIDERATIONS.

Chemical analyses of blood have for years been looked upon as belonging to experimental physiological chemistry, and, in no sense of practical use such as are urinary analyses, gastric contents analyses, etc. As bedside aids to diagnosis, blood chemical analyses did not really exist until the epoch-making work of Folin brought the question to the very forefront of medical literature. It was Folin who called attention to the practicability of making blood chemical tests with the idea in view of aiding the physician in diagnosis, using "microchemical" methods which have proved successful in quantitative analytical chemistry. His work has been followed by others who have simplified some of the methods. Such eminent authorities as Folin and Denis, Benedict and Lewis, and Myers and Fine deserve much credit for introducing these new and reliable methods of clinical laboratory technic.

It might be asked here, of what practical use is blood chemistry; what additional information can it give us over the tried and accepted methods of urinary analyses? Are the data obtainable from blood chemical manipulations of more service to the diagnostician than are urinary findings? Does blood chemistry give data not hitherto obtainable with urine chemical methods? We must emphatically answer "yes," to both questions. In fact, we trust that the reader will recognize, after the perusal of this book, that blood chemical analyses far surpass in value the most

exact and intricate qualitative and quantitative urinary analyses. We aim to convince the reader that of the two sets of facts, one furnished by urine analyses, the other, by blood analyses, the latter is of far greater importance. We do not wish to decry, for a moment, the carrying out of routine urinary analyses, nor do we wish to minimize the splendid helpfulness of a good urine analysis: rather, do we say that blood and urine investigations should go hand in hand, but that the information obtainable from the blood chemical analysis, being of a different character, representing estimation of *retained* products of metabolism rather than the estimation of pathologically changed ingredients of a fluid such as a search for albumin or sugar in urine implies, gives a far better idea of metabolic changes and furnishes a superior basis for the diagnostic and prognostic evaluation of a case to that furnished by the urine analyses. The blood chemical analysis tells us what the blood is storing up, what the kidneys are doing and what they are *not* doing, and also the exact status of nitrogenous and carbohydrate equilibrium. The urine analysis tells us a great deal about the pathology of the kidney function. One might be described as an estimation of the organic changes in the kidneys; the other, the blood chemical analysis, is an estimation of the *minutiae* of the renal function, from a pathological chemical and a pathological physiological viewpoint. Undue excretion of sugar in the urine is pathological, but how about the interpretation of the finding of glycosuria? We know that the amount of sugar in the blood gives a far better picture of carbohydrate metabolism than does the appearance of sugar in the urine. Sugar appears in the urine in a case of diabetes mellitus purely as an "overflow" proposition, whereas there may be an enormous sugar retention in the blood before the kidneys permit it to leak through. Thus an individual may have a hyperglycemia long before he has a glycosuria. There may be a so-called prediabetic stage to which the older writers often referred; only a blood chemical estimation of sugar would detect this. Again, there may be a case of low hyperglycemia and pronounced glycosuria with kidneys in individual cases readily permeable to sugar. Glycosuria in this case would give one no idea of the low grade of hyperglycemia. In renal diabetes, too, there is no hypergly-

cemia, simply a glycosuria possibly due to unusual permeability of the kidneys for the normal blood sugar, never a hyperglycemia. How could one differentiate then between diabetes mellitus and renal diabetes without a comparative blood and urine chemical analysis?

We feel that the subject has now been sufficiently worked out to demand a condensation of all the facts gleaned by blood chemistry and their interpretation in clinical medicine into a small textbook for the information of those who are interested. The literature has appeared practically in only the technical journals, principally the *Journal of Biological Chemistry*. These articles are, as a rule, inaccessible to many physicians and even to some of the laboratory workers in communities where there is no medical library. The writers' task is, therefore, to give fully the best methods that have been devised by the workers in this field together with such facts as they themselves have gleaned during years of effort, together with the most important literature on this question. The subject is under close investigation and rapid strides are being made. It, therefore, behooves those who are interested in the practical and scientific sides of medicine to keep informed on all this progress. We trust that our modest efforts will assist in spreading the facts before those not familiar with them and that others may be stimulated to assist in this work of accurately estimating bodily metabolism in health and in disease.

We shall, later on in the work, give our interpretation of the technical findings in blood and urine chemistry. Owing to the wide interest in this newborn side of laboratory diagnosis, we wish to immediately take up the question of installation of the laboratory for this sort of work and the actual technic of the tests.

Installation of the Blood and Urine Chemical Laboratory.

We have described in the following pages the various apparatus, reagents, glassware, etc., needed in this work. We shall, as it were, construct a model laboratory for the reader in which he may most profitably pursue these investigations. We shall not enumerate unnecessary apparatus, but shall endeavor to make the wants of the prospective worker as few as possible. Stately halls, marble columns, and lavish expenditure do not alone imply great work. Simplicity, modesty, coupled with untiring zeal and exact

observation, have given us what great advances medicine today has gained, and to that end we will construct a practical and inexpensive laboratory for those who contemplate launching into this department of laboratory medicine.

We will give the essentials of equipment and the ideal of their arrangement, allowing the ingenuity and particular facilities of each worker contemplating taking up this technic to work out his own arrangement of laboratory furniture, etc.

Selection of the Room.—Preferably a room should be selected

Fig. 1.—View of one side of chemical laboratory showing balance, dessicator, etc.

with good northern exposure for the accurate reading of the colorimeter. There should be a well protected place for the chemical balance, safe from sunlight and jarring. There should be, also, a firm block of wood arranged conveniently for the placing of the centrifuge. There should be running water in the room for two purposes; one for suction in running a Chapman pump, the other for obtaining water for a water-bath, cleaning glassware, etc. There should also be a chemical hood with the customary outlet for permitting vapors to escape. There should

also be a convenient, strongly constructed table for the microscope and balance. This in a general way covers the arrangement of the room for the larger articles. In addition to these features, there should be shelving for the accommodation of the reagent bottles, with drawers and cupboards for the storage of glassware, tubing, etc. The work table should be large enough to permit from two to six Bunsen burners to be placed in rows for the simultaneous heating of blood specimens.

Fig. 2.—View of another side of chemical laboratory showing Van Slyke's carbon dioxide apparatus and the urea apparatus set up and connected to the suction.

For the purpose of illustrating several views of a model laboratory, we call attention to Figs. 1 and 2 which show the CO_2 apparatus, chemical balance set up, desiccator, etc. In Fig. 3 is shown the blood chemical table proper, with running water in the middle of same, the Chapman suction pump, and the water-bath set up for uric acid estimations. It also shows the arrangement of the cylinders for urea estimation, a complete description of which will be found in the chapter on this subject (see p. 42).

Fig. 3.—Blood chemical table showing urea apparatus and water-bath used for the uric acid determinations.

Chemicals and Apparatus Used in the Newer Chemical Analysis of Blood and Urine.

It is essential to have a "Hellige" colorimeter which is described on page 133 and a balance which is accurate to one-tenth of a milligram.

CHEMICALS.

Urea N.

Urease, 10 gms.
 Mercuric iodide, 200 gms.
 Potassium iodide, 100 gms.
 Potassium hydroxide, 400 gms.
 Amyl alcohol, 100 c.c.
 Caprylic alcohol, 25 c.c.
 Hydrochloric acid, 500 gms.

APPARATUS.

Urea N.

6 Volumetric flasks (1000 c.c., 500 c.c., 250 c.c.), 2 of each.
 50 Test tubes about 200 mm. long and of diameter such that will slip into 100 c.c. graduate.
 2 Nests of beakers from 50 c.c. to 1000 c.c. capacity.
 1 Sulphuric acid wash bottle.
 6 Bunsen burners.
 6 Tripods.
 6 Pieces wire gauze, asbestos center.
 1 Thermometer.

CHEMICALS.

*Urea N—Cont'd**Uric acid.*

Acetic acid, 500 gms.
 Alumina cream, 250 gms.
 Potassium cyanide, 30 gms.
 Silver nitrate, 30 gms.
 Magnesium sulphate, 50 gms.
 Ammonium chloride, 100 gms.
 Ammonia (conc.), 500 gms.
 Uric acid (Kahlbaum), $\frac{1}{2}$ gm.
 Sodium tungstate, 100 gms.
 Hydrogen disodium phosphate,
 25 gms.
 Dihydrogen sodium phosphate,
 5 gms.
 Sodium carbonate, 500 gms.

Sugar.

Picramic acid, $\frac{1}{2}$ gm.
 Picric acid, 100 gms.

Creatine and Creatinine.

Potassium bichromate, 25 gms.
 Creatinine, $\frac{1}{2}$ gm.
 Sodium hydroxide, 500 gms.

CO₂ Combining Power of Plasma.

Phenolphthalein, 10 gms.
 Sulphuric acid, 500 gms.
 Mercury, 5 lbs.
 Caprylic alcohol, 30 c.c.

APPARATUS.

Urea N—Cont'd

- 3 Graduates, 100 c.c. (no lips), non-graduated.
- 3 Graduates, 100 c.c. (no lips), graduated.
- 4 Volumetric flasks (50 c.c.).
- 9 Pipettes, 5 c.c., 20 c.c., 25 c.c. (3 of each).
- 6 Two-hole rubber stoppers to fit graduates.
- 1 Twenty-four foot tubing to fit holes.
- 1 Suction pump.
- 1 Desiccator.
- 1 Wash bottle and connection.

Uric acid.

- 4 Cylinders, (100 c.c.). (Graduated.)
- 6 Casseroles, (375 c.c. capacity).
- 12 Stirring rods, (6 in.).
- 1 Water-bath.
- 6 Funnels, about 4 in. diameter.
- 100 Filter papers (for above funnels).
- 6 Centrifuge tubes, (15 c.c., conical).
- 1 Centrifuge.
- 8 Pipettes, 2 c.c. and 10 c.c.—four of each.
- 1 Wash bottle and connection (for hot water).

Sugar.

- 6 Sugar tubes, graduated to 20 c.c.
- 3 Pipettes, 1 c.c. and 3 c.c.

Creatine and Creatinine.

- 8 Graduates, 10 c.c., 25 c.c.—4 of each.
- 3 Pipettes, 1 c.c. graduated 1/100.
- 12 Centrifuge tubes, 15 c.c. and 50 c.c.—six of each.
- 1 Autoclave.

CO₂ Combining Power of Plasma.

- 1 Van Slyke apparatus.
- 1 Heavy stand and rod.
- 1 6-ft. Heavy suction tubing.
- 1 Iron rod and connection.

CHEMICALS.

*CO₂ Combining Power of Plasma—
Cont'd.*

Nonprotein Nitrogen.

Potassium sulphate, 50 gms.
Copper sulphate, 50 gms.
Trichloroacetic acid, 100 c.c.
Kaolin, 25 gms.

Cholesterol.

Chloroform, 500 c.c.
Acetic anhydride, 50 c.c.
Cholesterol or naphthol,
Green B, 1 gm.
Ether, 250 c.c.
Alcohol (redistilled), 500 c.c.

*Total Solids.**Chlorides.*

Colloidal iron, 50 c.c.
Potassium chromate, 25 gms.
Silver nitrate, 10 gms.
Ferric ammonium sulphate, 100 gms.
Nitric acid, 500 gms.
Ammonium thiocyanate, 10 gms.

Total Nitrogen.

Congo red, 5 gms.
Peroxide of hydrogen, 50 c.c.

Phenolphthalein.

Phenolsulphonphthalein in 1 c.c.
ampules—3.

Ammonia.

Included in foregoing.

APPARATUS.

*CO₂ Combining Power of Plasma—
Cont'd.*

- 1 Large clamp and connection.
- 2 Rings.
- 6 Dropping bottles (with rubber nipples).
- 1 Separating funnel.
- 1 Apparatus for saturating blood plasma (consisting of bottle filled with glass beads and connection).

Nonprotein Nitrogen.

- 3 Microburners.
- 1 Apparatus for removing fumes (large bottle, 2-hole rubber stopper and connection, 1 stand and connection).

Cholesterol.

- 3 100 c.c. graduated flasks.
- 3 25 c.c. beakers.
- 2 10 c.c. glass-stoppered, graduated cylinders.

Total Solids.

- 2 Weighing bottles (glass stoppers and block of filter and connection).

Chlorides.

- 3 Evaporating dishes, 50 c.c. capacity.
- 2 Volumetric flasks, 25 c.c. capacity.
- 2 Burettes, stand and connection.

Total Nitrogen.

- 2 Kjeldahl flasks.
- 1 Digestion rack, consisting of outlet for fumes, distilling outfit, and receiving bottle.

Phenolphthalein.

- 2 Graduates, 1000 c.c.
- 1 Accurately graduated 1 c.c. glass syringe with needles.

Ammonia.

Included in foregoing.

A high power centrifuge is advisable, one that can carry 15 c.c., 50 c.c., and 100 c.c. centrifuge tubes. Fig. 4 illustrates a convenient method of placing the centrifuge so as to economize space. The centrifuge is set on heavy blocks of wood so as to avoid undue vibration. The work table is hinged so as to utilize the space occupied by the centrifuge.

Fig. 4.—Showing a high power centrifuge placed so as to economize space.

Manner of Procuring and Handling of Blood.

The withdrawal of blood can best be accomplished by following the method of one of the writers (Gradwohl) in obtaining blood for the Wassermann reaction (see Fig. 5), which is as follows:

Expose the bend of the elbow where a prominent vein can usually be found. In women and men with a good deal of adipose tissue, these veins are sometimes not visible. In such cases, select the wrist or back of the hand. Place a tourniquet, either bandage or rubber tubing, above the bend of the elbow. The patient is then instructed to double his fist, which still further assists in

distending the veins between the fist and the portion of the arm upon which the tourniquet is tied.

The skin over the vein is then thoroughly cleansed by rubbing vigorously with alcohol. *Although iodine is a good antiseptic, it is not advisable to use it, as it leaves a dark stain on the skin which obscures the vein and makes it difficult to find.*

The needle is then removed from the test tube and plunged into the vein, procuring at least 25 c.c. of blood in this manner.

Fig. 5.—Manner of procuring blood.

At this point we might call attention to the usefulness of the Gradwohl tourniquet (Fig. 6) in blood withdrawal. This gives uniform compression and readily permits one to liberate the tourniquet without dislodging the needle from the vein. By alternately releasing and clamping the tourniquet, sufficient blood may be obtained by this means for a complete chemical analysis.

The blood should be taken in the morning, before breakfast. In other words, if it is not convenient to take the blood before the usual breakfast hour, then it may be taken later, but the patient

must not eat anything until after the blood is taken. The reason for this is that all data on the normal standards and the pathological changes have been obtained with blood obtained under these conditions. Therefore, for the sake of uniformity, we would recommend this method.

Amount of Blood Needed.—Twenty-five cubic centimeters of blood should be withdrawn for a complete analysis.

Fig. 6.—Gradwohl tourniquet.

This blood is allowed to run from the needle into small chemical bottles (see Fig. 7) containing 10 drops of 20 per cent solution of potassium oxalate. This oxalate should be previously dried in the oven over night at 100° C.

Fig. 7.—Chemical blood bottle.

As soon as possible after the 25 c.c. of blood have been obtained, one should quickly close the bottle and begin shaking vigorously so as to complete the defibrination of the blood which the potassium oxalate partially accomplishes. Do not stop shaking until perfect fluidity of the blood has been obtained. After defibrination of the blood, the process of chemical analysis should begin.

CHAPTER II.

SUGAR IN BLOOD.

It is advisable to begin the blood chemical analysis by estimation of sugar and creatinine first, because these two substances most quickly deteriorate and hence their estimation should be begun at once. Urea and uric acid determinations can be done later.

Take a 50 c.c. centrifuge tube (Fig. 8) and place in it 20 c.c. of distilled water. Suck up 5 c.c. of the blood into an Ostwald

Fig. 8.—50 c.c. centrifuge tube.

pipette (Fig. 9) and allow it to run into the bottom of the centrifuge tube, below the water. Wash the pipette by alternating drawing up and blowing down this blood and water mixture. Stir the mixture to lacerate the cells. Add 0.5 gram dry picric acid which precipitates the protein. Stir thoroughly. Allow it to stand 10 to 15 minutes. Stir occasionally. Place in centrifuge and run for 5 minutes at about 1500 revolutions per minute. Now remove the tube from the centrifuge and filter the mixture through a small filter paper into a clean, dry test tube. Part of this filtrate is used for the sugar estimation and part for the creatinine estimation. Take 3 c.c. of the filtrate for the sugar test, the remainder being reserved for the creatinine test. Place 3 c.c. of the filtrate in a sugar tube (Fig. 10); add 1 c.c. of saturated solu-

1

2

3

PLATE II.—STANDARD WEDGES.

1. Standard Picramic Acid Wedge.
2. Standard Bichromate (Normal) Wedge.
3. Standard Creatinine Wedge.

tion of sodium carbonate,¹ and mix. Immerse the test tube containing this mixture in a large beaker of water and then boil the beaker over a free flame for 15 minutes (Fig. 11), after which it is al-

Fig. 9.—Ostwald pipette.

Fig. 10.—Graduated sugar tube.

lowed to cool. The final step in the test is to so dilute this cooled solution with distilled water that it will be weaker in color than the standard picramic acid solution with which it is to be compared in the colorimeter. To this end we dilute it to 10, 15, or

¹This is prepared by dissolving 220 grams of anhydrous sodium carbonate in 1000 c.c. of distilled water.

20 c.c. [see marks upon the graduated sugar tube (Fig. 10)]. It must be remembered that in normal cases a dilution up to 10 c.c. will suffice, but beyond this it is often necessary to dilute to 15 c.c. or even 20 c.c. in cases of hyperglycemia. It is now compared

Fig. 11.—Showing sugar tube immersed in a beaker of water.

in the colorimeter with the wedge of standard picramic acid. (See Plate II for the color of the standard picramic acid wedge.)

The standard picramic acid solution is a staple solution and is made as follows: Dissolve 0.1 gm. picramic acid and 0.2 gm. anhydrous sodium carbonate in 30 c.c. warm distilled water and dilute to 1 liter.

Calculation using Nelson's Colorimeter
Example 1.—Now let us assume that the reading with the colorimeter was 52. If your dilution is 10, subtract 52 from 100 which equals 48. With a dilution of 10, multiply this by 0.002 which equals 0.096, which means 0.096% of sugar present. This would be a normal finding.

Example 2.—Let us assume that the reading is 41 and the dilution is 25. 41 from 100 equals 59. Multiply this by 0.005 which equals 0.295 (hyperglycemia). In other words, with a dilution of 10, multiply the difference between the reading and 100 by 0.002; if dilution is 15, multiply the difference by 0.003; if the dilution is 20, multiply by 0.004; if the dilution is 25, multiply by 0.005; etc.

Identical results may be obtained by using the data presented in Table I, providing the estimation was made on the basis of a dilution of 10. If it was diluted to 15 c.e., multiply the result by 1.5; to 20 c.e., multiply by 2; etc.

TABLE I²

ESTIMATION OF BLOOD SUGAR WITH HELLIGE COLORIMETER					
COLORIMETRIC READING	BLOOD SUGAR IN PER CENT	COLORIMETRIC READING	BLOOD SUGAR IN PER CENT	COLORIMETRIC READING	BLOOD SUGAR IN PER CENT
25	0.150	45	0.110	65	0.070
26	0.148	46	0.108	66	0.068
27	0.146	47	0.106	67	0.066
28	0.144	48	0.104	68	0.064
29	0.142	49	0.102	69	0.062
30	0.140	50	0.100	70	0.060
31	0.138	51	0.098	71	0.058
32	0.136	52	0.096	72	0.056
33	0.134	53	0.094	73	0.054
34	0.132	54	0.092	74	0.052
35	0.130	55	0.090	75	0.050
36	0.128	56	0.088	76	0.048
37	0.126	57	0.086	77	0.046
38	0.124	58	0.084	78	0.044
39	0.122	59	0.082	79	0.042
40	0.020	60	0.080	80	0.040
41	0.118	61	0.078	81	0.038
42	0.116	62	0.076	82	0.036
43	0.114	63	0.074	83	0.034
44	0.112	64	0.072	84	0.032

²Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

Sugar normal = 0.08 - 0.11 %

The authors wish to caution the beginner in this work to make his readings as quickly as possible as these colors deteriorate very rapidly, rendering a difference of from 1 to 3 points on the scale of the colorimeter.

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CHAPTER III.

CREATININE.

We begin this estimation by taking the remaining filtrate as already described in the sugar estimation, i.e., that part of the filtrate left after we took the 3 c.c. for the sugar test. Take 10 c.c. of this filtrate. (At this point we wish to emphasize the fact that in this test the unknown and the standard solution must be made up at the same time to prevent the development of the color in one case faster than that in the other, thereby obtaining incorrect results.) To the 10 c.c. filtrate add 0.5 c.c. of a 10% sodium hydroxide solution, and to 20 c.c. of the standard creatinine solution add 1 c.c. of 10% sodium hydroxide. (See Plate II for the color of the standard creatinine wedge.) Allow both to stand 10 minutes and read in the colorimeter.

TABLE II¹

ESTIMATION OF CREATININE IN THE BLOOD WITH THE HELBIGE COLOFIMETER

COLORI-METRIC READING	CREATININE MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	CREATININE MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	CREATININE MGMS. PER DILUTION OF 100 C.C.
40	0.80	57	0.55	74	0.31
41	0.78	58	0.54	75	0.30
42	0.77	59	0.52	76	0.28
43	0.75	60	0.51	77	0.27
44	0.74	61	0.50	78	0.25
45	0.72	62	0.48	79	0.24
46	0.71	63	0.47	80	0.22
47	0.70	64	0.45	81	0.21
48	0.68	65	0.44	82	0.20
49	0.67	66	0.42	83	0.18
50	0.65	67	0.41	84	0.17
51	0.64	68	0.40	85	0.15
52	0.62	69	0.38	86	0.14
53	0.61	70	0.37	87	0.12
54	0.60	71	0.35	88	0.11
55	0.58	72	0.34	89	0.10
56	0.57	73	0.32	90	0.09

¹The table here given must be used when N/4 bichromate is used as a standard. From Meyers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

The standard solution of creatinine is made by dissolving 15 mgms. of pure creatinine in 1000 c.c. of a saturated solution of picric acid.

The formula for the computation of this result is as follows:
 $89 \text{ minus reading} \times 0.0179 \times 5 = \text{mgms. of creatinine per 100 c.c. of blood.}$

Example.—Let us assume the reading in an experiment is 64. Then $89 \text{ minus } 64 = 25 \times 0.0179 = 0.4475 \times 5 = 2.2375 \text{ mgms. (normal).}$

Slightly less accurate results than these may be obtained by using N/4 bichromate of potash solution. (See Plate II for the color of the standard bichromate wedge.) When using this solution as a standard the filtrate is treated as in the preceding, and the result is multiplied by 5. The reader is referred to Table II (page 34) which should be used when N/4 potassium bichromate is used as a standard.

The standard potassium bichromate is made by dissolving 12.28 grams of potassium bichromate in distilled water and making up to 1 liter.

The authors recommend the pure creatinine over the latter method inasmuch as repeated experiences with the two methods give greater percentages of accurate findings with the former.

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CHAPTER IV.

CREATINE.

For the determination of creatine (and creatinine), pipette with an Ostwald-Folin pipette 1 or 2 c.c. of the remaining filtrate from the sugar estimation into a small test tube or 10 c.c. graduate, and autoclave at twenty pounds pressure for twenty minutes. At the end of this time cool the solution, make up to 8 c.c. with a saturated solution of picric acid, and then add 0.4 c.c. of a 10% sodium hydroxide solution. At this point it is also well to emphasize the fact that the unknown and the standard must be made up at the same time. To 20 c.c. of standard creatinine,¹ add 1 c.c. of a 10% solution of sodium hydroxide (this should be added at the same time the 0.4 c.c. is added to the unknown) and then compare the unknown and the standard after standing for ten minutes. The formula for computation of this result is as follows: 89 minus reading $\times 0.0179 \times 20 =$ mgms. creatinine and creatine.

Slightly less accurate results may be obtained by using N/4 potassium bichromate² as a standard.

If the accurate value of creatine is desired, this is obtained by subtracting the value of creatinine from the creatine and creatinine and multiplying it by 1.16.

Example.—Let us assume that the reading was 69. Then 89 minus 69 = $20 \times 0.0179 = 0.358 \times 20 = 7.16$ mgms. of creatinine + creatine = $7.16 - 2.2375$ (mgms. creatinine) = $4.9225 \times 1.16 = 5.7101$ mgms. creatine per 100 c.c. blood (normal).

¹This standard is made by dissolving 15 mgms. of pure creatinine and making up to one liter with saturated picric acid.

²This is prepared by dissolving 12.28 grams of potassium bichromate in distilled water and making up to 1000 c.c.

CHAPTER V.

URIC ACID.

Place 10 c.c. of blood in a casserole (Fig. 12) of at least 375 c.c. capacity. Add 50 c.c. of N/100 acetic acid.

The N/100 acetic acid is prepared by adding 0.6 c.c. glacial acid to 1 liter of distilled water.

This lasts about two weeks and should be cast aside after that time and a new solution made.

Place the casserole in a water-bath and heat until coagulation takes place. This usually takes about ten minutes with an effi-

Fig. 12.—Casserole.

cient water-bath. Heat the casserole over a free flame until it comes to a boil, stirring continuously. Now add about one spoonful (4 c.c.) of alumina cream. (For the preparation of alumina cream, take 500 c.c. of 8% aluminum acetate in acetic acid. This 8% solution may be purchased from any reliable chemical house. Precipitate this with sodium bicarbonate (dry) until the solution is neutral. This is verified by litmus paper estimation. Allow this to stand 24 hours and decant the supernatant fluid. This is repeated six times, that is, add distilled water and mix and allow to stand another 24 hours. In this way, it takes about six days to make this reagent. On the last day the precipitate is filtered and put in a jar, with the addition of 5 c.c. of chloroform. It is now ready for use. It should be kept in the ice-box for storage.)

Boil for one minute, stirring continuously. We now filter this solution and wash back the coagulum on the filter paper into

the casserole with about 100 c.c. of hot distilled water. Heat this mixture in the casserole over a free flame to the boiling point, and filter. Evaporate the combined filtrates down to 1 or 2 c.c. in the following manner. Boil slowly over a *free flame* until the volume has been reduced to about 50 c.c. Continue the evaporation in the *water-bath* down to 1 or 2 c.c. Transfer this to a conical centrifuge tube of 15 c.c. capacity, washing the casserole with two or three hot water portions. The final volume in the centrifuge tube should be kept *below* 10 c.c. When this has cooled, add fifteen drops of ammoniacal-silver-magnesium¹ mixture and the tube is shaken and placed in a refrigerator for about fifteen minutes (to allow for

Fig. 13.—Showing centrifuge tube attached to suction.

the precipitation of purine). Centrifuge the tube from three to five minutes, then invert, and pour off the supernatant fluid. Wipe the lip of the tube with filter paper and allow the ammonia to volatilize by suction. This is accomplished by attaching the centrifuge tube to the rubber tubing of the Chapman pump (Fig. 13).

We are now ready for the development of color and the reading. As before mentioned, the beginner should work as fast as possible as the color may fade or turbidity may develop. It is a general axiom, of course, that turbid solutions cannot very well be read in a colorimeter.

¹For the preparation of ammoniacal-silver-magnesium mixture, mix 70 c.c. of 3% silver nitrate solution, 30 c.c. of magnesium mixture, and 100 c.c. of concentrated ammonia. Any turbidity which may develop is removed by filtration. The magnesia mixture alluded to is made as follows: Dissolve 35 grams of magnesium sulphate and 70 grams of ammonium chloride in 280 c.c. of distilled water and then add 140 c.c. of concentrated ammonia.

Prepare a 100 c.c. graduated *cylinder* for the unknown and a 50 c.c. *volumetric flask* for the standard solution (Fig. 14). Then pipette 5 c.c. of uric acid standard² (5 c.c.=1 mgm. of uric acid) into the 50 c.c. volumetric flask. To the uric acid standard add two drops of a 5% solution of potassium cyanide, 2 c.c. of Folin-Macallum³ reagent, 20 c.c. of saturated sodium carbonate, and in one minute add water to the 50 c.c. mark. (See Plate I for the color of the standard uric acid wedge.) To the precipitate in the centrifuge tube add 2 drops of a 5% potassium cyanide solution (the tube

Fig. 14.—Volumetric flask.

is shaken so as to dissolve the precipitate), and 2 c.c. of the Folin-Macallum reagent, and then wash the contents of the centrifuge tube into a 100 c.c. graduate with from 15 to 20 c.c. of saturated sodium carbonate. If the color is developed well, use more carbonate, i. e., 20 c.c. when the color is stronger than the standard,

²For the preparation of standard uric acid solution, dissolve 9 gms. pure crystalline hydrogen disodium phosphate and 1 gm. dihydrogen sodium phosphate in 200 to 300 c.c. hot distilled water. Filter and make up to 500 c.c. with hot water. Pour this warm, clear solution on 200 mgms. pure dried uric acid (Kahlbaum) suspended in a few cubic centimeters of water in a liter volumetric flask. Agitate until completely dissolved, add at once exactly 1.4 c.c. glacial acetic acid. Make up to one liter, mix and add 5 c.c. chloroform. 5 c.c. of this solution are equivalent to 1 mgm. of uric acid. This solution should be freshly prepared once every two months. Before weighing out the 200 mgms. of uric acid it is well to dry overnight the quantity from which the measure is to be made in a drying oven at 100° C.

³For the preparation of Folin-Macallum reagent, boil 100 gms. of sodium tungstate, 20 c.c. concentrated hydrochloric acid, and 30 c.c. of 85% phosphoric acid in 750 c.c. distilled water for two hours and then make up to 1000 c.c. with distilled water. In boiling, it is well to have a funnel over the flask so as to prevent undue evaporation.

and 15 c.c. when it is weaker. The fundamental principle of these dilutions in microchemical work is to have the unknown solution weaker in color than the standard solution. A period of time of from forty to sixty seconds should be allowed to elapse before determining whether to dilute to 50 or 100 c.c. Dilute with distilled water to 25, 50, or 100 c.c., depending upon the depth of color obtained. Table III gives the data for working out the amount of uric acid present.

Example.—Suppose the final dilution of the unknown was 25 and the reading was 42. 42 in the table is equivalent to 1.24 mgms. This is divided by 4 because it is $\frac{1}{4}$ as strong as the amount in the table (i.e., $\frac{1}{4}$ of 100) which equals 0.31 mgms. in 10 c.c. of blood (which is the amount of blood we started with). In 100 c.c. of blood we would have $10 \times 0.31 = 3.1$ mgms.

TABLE III⁴

ESTIMATION OF URIC ACID WITH HELBIGE COLORIMETER					
COLORIMETRIC READING	URIC ACID MGMS. PER DILUTION OF 100 C.C.	COLORIMETRIC READING	URIC ACID MGMS. PER DILUTION OF 100 C.C.	COLORIMETRIC READING	URIC ACID MGMS. PER DILUTION OF 100 C.C.
20	1.67	40	1.28	60	0.88
21	1.65	41	1.26	61	0.86
22	1.63	42	1.24	62	0.84
23	1.61	43	1.22	63	0.82
24	1.59	44	1.20	64	0.81
25	1.57	45	1.18	65	0.79
26	1.55	46	1.16	66	0.77
27	1.53	47	1.14	67	0.75
28	1.51	48	1.12	68	0.73
29	1.49	49	1.10	69	0.71
30	1.48	50	1.08	70	0.69
31	1.46	51	1.06	71	0.67
32	1.44	52	1.04	72	0.65
33	1.42	53	1.02	73	0.63
34	1.40	54	1.00	74	0.61
35	1.38	55	0.98	75	0.59
36	1.36	56	0.96	76	0.57
37	1.34	57	0.94	77	0.55
38	1.32	58	0.92	78	0.53
39	1.30	59	0.90	79	0.51

⁴Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

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- # 100-100
Blau
X
u.

CHAPTER VI.

UREA.

Into a test tube that will readily slip into a 100 c.c. graduated cylinder introduce 2 c.c. of distilled water and 0.1 gm. of urease,¹ and 2 c.c. of blood with an Ostwald pipette; then incubate the tube in a beaker of water at 50° C. for one-half hour. At the end of this time add two drops of caprylic alcohol or 1 c.c. of amylic alcohol to prevent foaming in aeration.

We now direct our attention to the manner of setting up the glassware for the continuation of this test. The chemistry of this estimation is about as follows: The enzyme urease converts urea into ammonium carbonate. The ammonia is then liberated by aeration in the presence of sodium carbonate in excess and goes over into the hydrochloric acid as ammonium chloride. This can be determined colorimetrically by the use of Nessler's reagent. There should be two cylinders for each sample of blood. If more than one specimen of blood is to be examined, these cylinders may be run in series, two for each test. One cylinder is graduated, the other nongraduated. Fig. 15 shows the manner of arranging this glassware.

A two-hole rubber stopper is placed in each cylinder. Cylinder 1 (*A-A'*) is graduated and is connected with the suction. Cylinder 2 (*B-B'*) is nongraduated and is connected with the acid wash (*C*) bottle. This acid wash bottle is simply a bottle containing sulphuric acid (10%) placed at the end of the outfit to prevent the ammonia in the air from gaining entrance into the test. Cylinder 1 (*A-A'*) has a short tube bent at right angles connected to the suction and only extending in the cylinder to a point just within the cylinder. This is tube *F-F'*. Tube *G-G'* extends almost to the bottom of cylinder 1. It has a sealed ending with small holes punched in its side. This can readily be done as follows: The holes may be made with a platinum wire which is at white heat, provided the glass is only moderately hot. Cylinder 2 has a right-

¹Urease may be purchased from the Arlington Chemical Co., Yonkers, N. Y.

Fig. 15.—Showing the urea apparatus set up and connected to suction.

angle tube extending to a point just below the stopper (*D*). It has another tube with a straight open end dipping into the test tube (*E*) and running out to be connected either with the acid wash bottle extension or with another series of cylinders in case more than one specimen of blood is under examination.

Into the 100 c.c. graduated cylinder (cylinder 1) place 20 c.c. distilled water and two to three drops of 10% hydrochloric acid. Now close cylinder 1 and open cylinder 2. To the test tube containing the digested blood allow an equal volume of saturated sodium carbonate to slowly run down under the blood. Immediately and carefully insert the tube into cylinder 2 and immediately close, and then carefully and tightly seal the connection. The suction is started by means of the Chapman pump, the rate is slow for about five minutes and then gradually increased as much as the apparatus will stand. The aeration is kept up from thirty to forty-five minutes. At the end of this time, disconnect the tube and use cylinder 1 for the final determination. Remove the rubber stopper from cylinder 1 and wash the tube with distilled water (2 to 3 c.c.).

We now come to the development of color. Into a 50 c.c. volumetric flask pipette 5 c.c. of ammonium sulphate solution containing 1 mgm. of nitrogen (this is the standard solution), add 25 c.c. distilled water, and then 20 c.c. Nessler's solution, diluted 1 to 5. (See Plate I for the standard color of 1 mgm. of nitrogen.)

The standard ammonium sulphate solution is prepared as follows:

Dissolve 0.944 gm. ammonium sulphate of the highest purity in distilled water and make up to 1000 c.c. in a volumetric flask.

Nessler's solution is prepared as follows: for one liter we need:

Mercuric iodide	100 gms.
Potassium iodide	50 gms.
Potassium hydroxide	200 gms.

Place the mercuric iodide and the potassium iodide, both finely powdered, into a liter volumetric flask and add about 400 c.c. distilled water. Now dissolve the potassium hydroxide in 500 c.c. distilled water, cool thoroughly, and add with constant shaking to the mixture in the flask. Then make up to one liter with water. This usually becomes perfectly clear. Keep at 37° C. in incubator over night or until

the yellowish white precipitate which may settle out is thoroughly dissolved and only a small amount of dark brownish red precipitate remains. The solution is now ready to be siphoned off and used.

To cylinder 1 containing the unknown in the form of ammonium chloride, add from 10 to 20 c.c. of diluted Nessler's solution (1 to 5), dependent upon the depth of color, and then dilute to 50 c.c., 100 c.c., etc., depending upon the color. The colorimetric reading should be made at once and computed from the following table:

TABLE IV²

ESTIMATION OF NITROGEN WITH THE HELIGE COLORIMETER					
COLORI-METRIC READING	NITROGEN MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	NITROGEN MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	NITROGEN MGMS. PER DILUTION OF 100 C.C.
20	1.73	40	1.31	60	0.89
21	1.71	41	1.29	61	0.87
22	1.69	42	1.27	62	0.85
23	1.67	43	1.25	63	0.83
24	1.65	44	1.23	64	0.81
25	1.62	45	1.20	65	0.78
26	1.60	46	1.18	66	0.76
27	1.58	47	1.16	67	0.74
28	1.56	48	1.14	68	0.72
29	1.54	49	1.12	69	0.70
30	1.52	50	1.10	70	0.67
31	1.50	51	1.08	71	0.65
32	1.48	52	1.06	72	0.63
33	1.46	53	1.04	73	0.61
34	1.44	54	1.02	74	0.59
35	1.41	55	0.99	75	0.56
36	1.39	56	0.97	76	0.54
37	1.37	57	0.95	77	0.52
38	1.35	58	0.93	78	0.50
39	1.33	59	0.91	79	0.48

²Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

Example.—Suppose the dilution was to 50 and our reading 75. 75 on our scale is equivalent to 0.56 mgms. Divide this by 2 because our dilution was to 50, which is one-half of 100, which will give us 0.28 mgms. in 2 c.c. of blood. In 1 c.c. of blood we would

have 0.14 mgms. of urea nitrogen and in 100 c.c. of blood we would have 14 mgms., which is about normal.

Should it be desired to convert this urea nitrogen into urea, the results are always multiplied by the factor 2.14.

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CHAPTER VII.

NONPROTEIN NITROGEN.

In a 50 c.c. volumetric flask with about 35 c.c. of 2.5% trichloroacetic acid, add 5 c.c. of blood, and make the volume up to 50 c.c. with 2.5% trichloroacetic acid. Shake the flask vigorously, and at the end of 30 minutes (or as soon after as convenient) filter the solution through a dry filter. To the filtrate add about two grams of kaolin, and shake the solution vigorously. After allowing the mixture to stand for a few minutes (5 to 10), filter again. The filtrate should now be quite colorless. Pipette 10 c.c. of the filtrate (the equivalent of 1 c.c. of blood) into a test tube about 200 mm. long and of a sufficient diameter to slip into a 100 c.c.

Fig. 16.—Microburner.

graduated cylinder (no lip). Then add one-tenth to three-tenths of a gram of potassium sulphate, a drop of 10% copper sulphate, and 1 c.c. of concentrated sulphuric acid in the order named (these reagents should be of the highest purity). This is then boiled over a microburner (Fig. 16), at first gently, until a dark brown color appears.

At this point it might be well to call the attention of the reader to a modification of this test¹ which will serve for blood as well as urine estimations, and which will serve to shorten this test about ten minutes. Allow the solution to cool and add a drop of peroxide of hydrogen. If the mixture does not clear, heat gently over the microburner. Repeat this process once more if the mixture is not perfectly clear (digested). One drop of peroxide of hydrogen will usually suffice. Now allow the tube to cool for a few minutes and then add about 5 or 6 c.c. of distilled water.

¹Gradwohl and Blaivas: Jour. Am. Med. Assn., Sept. 9, 1916, vol. lxvii, p. 809.

As a means of removing fumes, the suction is connected by a two-hole stopper to a large bottle containing a solution of sodium hydroxide (Fig. 17). The short tube A, bent at right angles, should be connected to the suction. The tube B should be attached to a

Fig. 17.—Apparatus for removing fumes in connection with nitrogen determinations.

funnel over the mouth of the test tube D. After a few determinations have been made, it is well to wash the funnel to remove any acid which may have condensed upon it.

Aeration is carried out exactly in the manner as for urea, only that saturated sodium hydroxide is used instead of saturated sodium carbonate. The same table² is also used for calculation and the results obtained for 1 c.c. of blood.

$$X = S \times \frac{\text{Tit}}{\text{SP.}}$$

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²See Table IV, p. 45.

CHAPTER VIII.

CHOLESTEROL.¹

Preparation of Sample.—Run 2 c.c. of whole blood, plasma, or serum slowly (a slow stream of drops) from a pipette into about 75 c.c. of a mixture of redistilled alcohol and ether (3 parts alcohol, 1 part ether) in a 100 c.c. graduated flask. Keep the contents of the flask in motion during the process so that there is no clumping of the precipitated material. Raise contents of the flask to boiling by immersion in a water-bath (with constant shaking to avoid superheating), cool to room temperature, fill to the mark with alcohol-ether, mix and filter. The filtered liquid if placed in a tightly stoppered bottle in the dark will keep unchanged for a considerable time so that, if it is not convenient to complete the determination at once, the sample may be carried to the above stage and left to a more suitable time.

By running the blood slowly into the large quantity of alcohol-ether, as above, the protein material is precipitated in finely divided form and under these conditions the short heating combined with the great excess of solvent is adequate for complete extraction of serum or plasma. The extraction, while not so complete in the case of whole blood, is believed to be better, because of the higher values obtained than that obtained by any other method in use at the present time.

Determination.—Measure 10 c.c. of the alcohol-ether extract into a small flat-bottomed beaker and evaporate *just* to dryness over a water-bath or electric stove. Any heating, after dryness is reached, produces a brownish color which passes into the chloroform and renders the subsequent determination difficult or impossible. The cholesterol is extracted² from the dry residue by boiling out three or four times with successive small portions of chloroform and decanting into a 10 c.c. glass stoppered, gradu-

¹Bloor: Jour. Biol. Chem., 1916, vol. xxiv, p. 229.

²In order to get an adequate extraction with the small amounts of chloroform used, an excess (3 c.c.) should be added each time and the mixture allowed to boil down to half its volume or less, before decanting.

ated cylinder. The combined extracts after cooling (5 c.c. or less) are then made up to 5 c.c. The solution should be colorless but not necessarily clear, since the slight turbidity clears up on adding the reagents.

To this solution add 2 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulphuric acid and after mixing place in the dark

TABLE V³

ESTIMATION OF CHOLESTEROL WITH THE HELLIGE COLORIMETER					
COLORI-METRIC READING	CHOLESTEROL MGMS. DILUTION OF 5 C.C.	COLORI-METRIC READING	CHOLESTEROL MGMS. DILUTION OF 5 C.C.	COLORI-METRIC READING	CHOLESTEROL MGMS. DILUTION OF 5 C.C.
15	0.74	35	0.57	55	0.40
16	0.73	36	0.56	56	0.40
17	0.72	37	0.55	57	0.39
18	0.71	38	0.55	58	0.38
19	0.70	39	0.54	59	0.37
20	0.69	40	0.53	60	0.36
21	0.69	41	0.52	61	0.35
22	0.68	42	0.51	62	0.35
23	0.67	43	0.50	63	0.34
24	0.66	44	0.50	64	0.33
25	0.65	45	0.49	65	0.32
26	0.65	46	0.48	66	0.31
27	0.64	47	0.47	67	0.30
28	0.63	48	0.46	68	0.30
29	0.62	49	0.45	69	0.29
30	0.61	50	0.45	70	0.28
31	0.60	51	0.44	71	0.27
32	0.59	52	0.43	72	0.26
33	0.59	53	0.42	73	0.25
34	0.58	54	0.41	74	0.24

³This table is good for both standards given above (cholesterol and Naphthol Green B). Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

for 10 minutes to allow for the development of the color. Then compare in the colorimeter (Hellige) with a standard cholesterol solution upon which the color is developed in the same way.⁴ See Plate I for the standard color of cholesterol.

⁴For the preparation of standard with pure cholesterol, pipette 2 c.c. of an 0.08% freshly prepared chloroform solution of cholesterol into a dry, accurately graduated 25 c.c. cylinder and make up to 10 c.c. with chloroform and add 4 c.c. acetic anhydride and 0.2 c.c. of concentrated sulphuric acid. Care should be taken that the unknown and the standard are made together and both the colors should be allowed to develop at the same time. The reason for this is that the colors fade rather rapidly. It is very important that the wedge and the cup of the colorimeter be perfectly dry.

An aqueous solution of Naphthol Green B⁵ can also be used as a standard. The cholesterol in 0.2 c.c. of blood, serum, or plasma, can be obtained from Table V. This table is suitable for both standards (pure cholesterol or Naphthol Green B).

The result multiplied by 500 will give the percentage of cholesterol.

Example.—Reading is 60 which equals 0.36 mgms. cholesterol in 0.2 c.c. blood, plasma, or serum. $0.36 \times 500 = 180$ mgms. or 0.18%.

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⁵For the preparation of Naphthol Green B, dilute 2 c.c. of a 0.1% aqueous solution of the dye to 17 c.c. with distilled water. The diluted solution appears to keep for a little time, while the concentrated solution apparently will keep for a considerable time. The permanency of the solution and the fact that the color is practically identical with that obtained from cholesterol makes the standard very convenient. Myers and Fine have found this solution nearly identical with the pure cholesterol standard. They advise, however, that in preparing a new solution it is best to standardize it by plotting a new curve.

CHAPTER IX.

TOTAL SOLIDS.

For the determination of total solids, a weighing bottle with a glass stopper and a glass loop (Fig. 18), which goes inside of the bottle when stoppered, to which a block of filter paper is fastened, is required.¹ From an accurately graduated pipette, allow 0.3-0.6 gms. of blood to flow rapidly on the filter paper. Quickly insert the stopper to prevent any loss of moisture, weigh the bottle. Tilt the stopper, and then place the bottle in a drying

Fig. 18.—Weighing bottle for total solids.

oven at 105° C. overnight. Whenever convenient, the bottle is cooled in the desiccator (care being taken that the stopper is closed) and again weighed. From the loss of moisture the total solids may be calculated.

Calculation.—Divide the weight of the residue by the weight of the blood used. The quotient is the percentage of solids contained in the blood examined.

¹Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

CHAPTER X.

TOTAL NITROGEN.

Place exactly 1 c.c. of blood in a long-necked Jena glass Kjeldahl flask (Fig. 19), add 20 c.c. of concentrated sulphuric acid and about 0.2 grams of copper sulphate, and boil the mixture in the

Fig. 19.—Kjeldahl flask.

digestion rack (Fig. 20) for some time after it is colorless (about one hour). Allow the flask to cool and dilute the contents with about 200 c.c. of ammonia-free water. Add a little more of a saturated sodium hydroxide solution than is necessary to neutralize the sulphuric acid (about 40 c.c.). Introduce into the flask a little coarse pumice stone or a few pieces of granulated zinc

to prevent lumping, and a small piece of paraffin to lessen the tendency to froth. By means of a safety tube connect the flask with a condenser (Fig. 21) so arranged that the delivery tube passes into a vessel containing a known volume (the volume used

Fig. 20.—Digestion rack.

depending upon the nitrogen contents of the blood) of $N/10$ sulphuric acid to which has been added a few drops of congo red,¹ care being taken that the end of the delivery tube reaches beneath the surface of the fluid. This delivery tube should be of a

Fig. 21.—Kjeldahl apparatus showing condenser.

large caliber in order to avoid the sucking back of the fluid. Mix the contents of the distillation flask very thoroughly by shaking (or rotating) and distil the mixture until about two-thirds of the solution has passed over. Titrate the partly neutralized $N/10$

¹0.5 gm. of congo red in a mixture of 90 c.c. of distilled water and 10 c.c. of 95% alcohol.

sulphuric acid against N/10 sodium hydroxide.² Calculate the amount of nitrogen in 1 c.c. of blood and multiply by 100 to report for 100 c.c. of blood.

Calculation.—1 c.c. of N/10 sulphuric acid is the equivalent of 0.0014 gm. nitrogen. (Preparation of N/10 NaOH and N/10 H_2SO_4 .)

Folin-Farmer Microchemical Method.

Pipette exactly 1 c.c. of the blood into a 25 c.c. volumetric flask. Then dilute with distilled water up to 25 c.c. Now pipette 1 c.c. of the diluted blood into a test tube of such a size that it will slip into the aeration apparatus (Fig. 15). Add one to three-tenths of a gram of potassium sulphate, a drop of 10% copper sulphate solution, and 1 c.c. of concentrated sulphuric acid in the order named, and carry out digestion as in the determination of non-protein nitrogen. (See page 47.) The result obtained above is for 1/25 c.c. of blood.

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²For the preparation of N/10 sodium hydroxide, dissolve 4 gms. of sodium hydroxide in about 900 c.c. of distilled water. Titrate this against a decinormal solution of oxalic acid which is made by dissolving exactly 6.285 gms. of pure oxalic acid in a liter of distilled water. The decinormal sodium hydroxide was purposely made too strong; therefore, less than 10 c.c. of the alkali will be required to neutralize 10 c.c. of the decinormal oxalic acid solution. Suppose that 9.5 c.c. of the alkali only were required, then every remaining portion of 9.5 c.c. of the unknown would have to be diluted with 0.5 c.c. of distilled water. This solution will contain the equivalent of one-tenth of its molecular weight in grams (4 grams) in 1000 c.c. of distilled water. From this N/10 alkali, N/10 HCl may be prepared.

CHAPTER XI.

CHLORIDES.¹

Pipette 3 c.c. of blood into a 50 c.c. graduated centrifuge tube (Fig. 22), then add 15 c.c. of N/100 acetic acid and dilute the volume to 30 c.c. with distilled water. Place the tube in a beaker of boiling water to bring about the coagulation of the protein, care being taken that the contents of the tube are agitated occasionally with a stirring rod. After the protein has coagulated, the tube is cooled, again made to volume (30 c.c.), and centri-

Fig. 22.—Graduated centrifuge tube.

fuged. After this is done, pour the slightly colored supernatant fluid into a dry centrifuge tube and add about six drops of a strong solution of colloidal iron and place the tube in a beaker of hot water for a few minutes. This brings about a complete precipitation of all protein. After centrifuging (or filtering) the clear fluid once more, pour it from the tube and take 10 c.c. (equivalent of 1 c.c. of blood) into a 50 c.c. evaporating dish or a 25 c.c. volumetric flask, depending on the method used, and titrate.

“Theoretically the Volhard-Arnold is to be preferred, but the substances which may interfere with the Mohr titration are so small that the results are practically identical. The former method is of advantage, however, when for any reason the fluid to be titrated has been rendered acid.”

Volhard-Arnold Method.

Pipette 10 c.c. of the filtrate into a 25 c.c. volumetric flask. Add 10 c.c. of the standard silver nitrate solution² (1 c.c. = 0.001 gm. of sodium chloride) and 1 c.c. of the ferric alum indicator,³

¹Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

²This standard is prepared by dissolving 2.906 gms. of silver nitrate in distilled water and making up to 1 liter.

³The indicator is made by dissolving 100 gms. of crystalline ferric ammonium sulphate in 100 c.c. of 25% nitric acid.

and finally make up to volume and shake thoroughly. Centrifuge this in a large (50 c.c.) centrifuge tube and decant the clear supernatant fluid. Titrate 20 c.c. of the fluid, which is the equivalent⁴ of 0.8 c.c. of blood, with a standard ammonium thiocyanate solution of the same strength as the silver nitrate, until a distinct yellow color shows throughout the mixture. The titration result, divided by 0.8, subtracted from 10, to obtain the silver nitrate used, and multiplied by .001, and again multiplied by 100 gives the percentage of chlorides as sodium chloride.

Example.—Reading on burette is 3.2 c.c. Divide by $0.8 = 4$; subtract from $10 = 6$; multiply by $0.001 = 0.006$ (gms. of NaCl in 1 c.c. of blood); multiply by 100 = 0.6% (normal).

Mohr Method.

Pipette 10 c.c. of the filtrate into an evaporating dish of 50 c.c. capacity and add one drop of a 10% solution of potassium chromate. Now run the standard silver nitrate (same as above, 1 c.c. equals 0.001 gm. of sodium chloride) into the dish from a burette until the first permanent precipitate of silver chromate, which is an orange-red color, shows throughout the whole solution on stirring. This is the end of the titration, for which there is a correction of 0.2 to 0.3 of 1 c.c. This result multiplied by 0.001, multiplied by 100 gives the percentage of chlorides as sodium chloride.

Example.—Reading on burette is 6.3 c.c. Subtract 0.3 c.c., equals 6 (corrected reading); multiply by 0.001 equals 0.006 (gms. of NaCl in 1 c.c. of blood); multiply by 100 equals 0.6% (normal).

⁴Standard ammonium thiocyanate is prepared by dissolving 1.3 gms. of ammonium thiocyanate in 800 c.c. of water, titrating against the above silver nitrate standard, and ascertaining the amount of water which must be added to the solution to make it equivalent to 1 c.c. of the standard silver nitrate solution or 0.001 gm. of sodium chloride.

CHAPTER XII.

TEST FOR ACIDOSIS IN BLOOD.

Van Slyke Method for the Determination of the Carbon Dioxide Combining Power of Blood Plasma.

Having centrifuged the fresh oxalated blood, pipette off the clear plasma and place in a separatory funnel of about 300 c.c. capacity. Slight hemolysis does not affect results appreciably, but hemolysis should be avoided as much as possible by immediate centrifugalization. In order to determine its alkaline reserve, saturate the plasma with carbon dioxide at alveolar tension. In other words, the operator blows vigorously through a bottle containing glass beads into the separatory funnel, as shown in Fig. 23. If one blows directly into the separatory funnel, enough moisture condenses on the walls of the funnel to appreciably dilute the plasma. Close the funnel at stop-cock *S* and stopper *T* just before the stream of breath stops, and shake for one minute in such a manner that the plasma is distributed as completely as possible about the walls. After the shaking has lasted a minute, blow a fresh portion of the alveolar air through the beads into the funnel and shake for one minute.

The CO_2 (Fig. 24) apparatus is held in a strong clamp *W*, which is lined with rubber, and the lower stop-cock is supported by an iron rod, which is also covered with soft rubber tubing. The apparatus is completely filled with mercury. Care should be taken that capillaries *A* and *F*, which are above the upper stop-cock, are also filled with mercury. There should be no air bubbles within the apparatus. Six dropping bottles, which contain the following solutions, should be at hand (see Fig. 25):

1. Distilled water.
2. Phenolphthalein (1% in 95% alcohol).
3. Normal ammonium hydroxide.
4. Caprylic alcohol.
5. Normal sulphuric acid.
6. Mercury.

Fig. 23.—Showing operator saturating blood plasma with carbon dioxide.

Fig. 24.—CO₂ apparatus.

The mercury leveling bulb *H* should be hung by wire *I* on extension *N*, about on the level with the lower cock *J*. The apparatus must be thoroughly cleaned before the determination is started. The apparatus can be tested by allowing the mercury to run down and then forcing it up by raising and lowering bulb *H*. The air is forced out and the mercury is caught in a bottle as shown in Fig. 26. (This is done until there is not a single air bubble in the apparatus.) Add one drop of phenolphthalein to the upper cup *B* and a drop or two of the ammonium hydroxide. Now dilute this with about $\frac{1}{2}$ c.c. of distilled water and draw off all except about two drops of the alkaline solution.

Now introduce 1 c.c. of the saturated plasma into the cup and allow it to flow under the alkaline solution, so that none of the

Fig. 25.—Dropping bottles for use in connection with CO_2 determination.

carbon dioxide escapes. Turn stop-cock *C* so that *E* and *Z* are connected and allow the plasma to run in until capillary *F* is exactly filled. Add 0.5 c.c. of distilled water to cup *B* and then allow to run down to capillary *F*. Repeat this, taking care that no air enters the apparatus with the liquid. Now admit into capillary *F*, 1 drop of caprylic alcohol to prevent foaming, and pour about 1.5 c.c. of the sulphuric acid into the cup. Admit enough of the acid into the apparatus, carrying the caprylic alcohol along with it, so that the total volume in the apparatus is exactly to the 2.5 c.c. mark. Draw off the excess sulphuric acid. Now place a few drops of mercury in cup *B* and allow to flow down to capillary *F*, in order to seal same and make it capable of holding an absolute vacuum. During this whole operation, the lower stop-cock *J* should remain open, and when the apparatus is set up, it should be

in such adjustment that, if the wire *I* which is connected to bulb *H* is lowered to hook *O*, the mercury will run to the mark *X* on the figure (Fig. 27), care being taken that the mercury will not run into fork *Y*. Place wire *I* on hook *O* and allow the mercury to fall until the meniscus of the mercury has dropped to the 50 c.c. mark on the apparatus. This is controlled by stop-cock *J*. The bubbles of CO_2 are now seen escaping.

In order to completely extract the carbon dioxide, remove the apparatus from the clamp and shake by turning it upside down about a dozen times. (The thumb should be placed over cup *B* so as not to lose any of the mercury.) Then replace the apparatus, the mercury leveling bulb *H* still being at the low level *O*, and allow the solution to flow into the small bulb below the lower stop-cock (right side). Drain the solution out of the portion of the apparatus above the stop-cock *J* as completely as possible, but without removing any of the gas (the last drop being allowed to remain above). Now raise the mercury bulb *H* in the left hand, and with the right hand immediately turn the lower stop-cock *J*, so that the mercury is admitted to the upper part of the apparatus through the left-hand entrance of the stop-cock without readmitting the watery solution. Hold the leveling bulb *H* beside the apparatus so that its mercury level corresponds to that in the apparatus, and the gas in the latter is under atmospheric pressure. A few hundredths of a cubic centimeter of water will float on the mercury in the apparatus, but this may be disregarded in leveling. The calculation of the result into terms of volume percentage of carbon dioxide, bound as carbonate by the plasma, is quite complicated and we consequently use the direct reading from the apparatus, minus .12.

Plasma of normal adults yield 0.65 c.c. to .90 c.c. of gas which is the direct reading on the apparatus. If .12 were subtracted, the normal figures would be 53 to 78 in terms of volume per cent of carbon dioxide chemically bound by the plasma. Figures lower than 50 per cent in adults indicate acidosis. The exact calculation of the result into terms of carbon dioxide bound as carbonate by the plasma is quite complicated and consequently the worker is advised to subtract .12 from his reading on the apparatus. The result thus obtained gives approximately (within 2 to

Fig. 26.—CO₂ apparatus showing air being forced cut.

Fig. 27.—CO₂ apparatus. Mercury should not go below mark X.

3 per cent) the volume per cent of carbon dioxide bound by the plasma.

Example.—Reading on the Van Slyke apparatus is 0.74 minus 0.12 which equals 0.62 per cent of carbon dioxide bound by 1 c.c. of plasma. For 100 c.c. of plasma multiply 0.62% by 100, which equals 62% (normal).

Marriott, Levy, and Rowntree Method for the Determination of the Hydrogen-ion Concentration of the Blood.

Principle of the Method.—Levy, Rowntree, and Marriott¹ state that the indicator method has not proved of great value in the studies of hydrogen-ion concentration of the blood, although the reaction of inorganic solutions may be determined accurately by this means.² Different indicators show their color changes at varying degrees of hydrogen-ion concentration: for example, the color of methyl orange changes from pink to yellow as the pH of its solution changes from 3 to 5. At intermediate points, various colors may be obtained and a certain color indicates a definite pH. Similarly, phenolphthalein changes from colorless to pink between pH8 and pH10 and can be used for the measurement of H-ion concentrations between these two points. In carrying out the indicator method, it is necessary to have a series of standard solutions of known pH and an indicator exhibiting easily distinguishable color changes at hydrogen-ion concentrations approximating that of the solution under consideration. It is then simply necessary to add equal amounts of indicator to the standard solutions and to the solution being tested and to determine which of the colors in the standard solutions most closely matches that of the unknown solution.

This method has been successfully used on the urine by Henderson and by Walpole. As proteins interfere with the colors of many indicators, and as both blood and serum possess color, it has been impossible to apply the method directly to the blood.

It seemed probable that the indicator method might be utilized for blood, provided coloring matters and proteins could be excluded by means of dialysis. If blood is dropped into collodion sacks

¹Levy, Rowntree, and Marriott: *Arch. of Int. Med.*, 1915, vol. xvi, p. 389.

²Sörenson: *Ergebn. d. Physiol.*, 1912, vol. xii, 393. A full description of indicators as used for this purpose.

and dialyzed for five minutes, the dialysate is free from proteins and coloring matter, but contains salts, and is well adapted to the use of indicators.

Since phenolsulphonphthalein exhibits definite variations in quality of color, with very minute differences in hydrogen-ion concentration between pH6.4 and 8.4, it was adopted as the indicator in this method.

Preparation of Standard Colors.—Standard phosphate mixtures are prepared according to Sörenson's directions as follows:

One-fifteenth Molecular Acid or Primary Potassium Phosphate.—Dissolve 9.078 grams of the pure recrystallized salt (KH_2PO_4), in freshly distilled water and make up to one liter.

One-fifteenth Molecular Alkaline or Secondary Sodium Phosphate.—Expose the pure recrystallized salt ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) to the air for from ten days to two weeks, protected from dust. Ten molecules of water of crystallization are given off and a salt of the formula $\text{Na}^2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ is obtained; dissolve 11.876 grams of this in freshly distilled water and make up to one liter. The solution should give a deep rose red color with phenolphthalein. If only a faint pink color is obtained, the salt is not sufficiently pure.

Mix the solutions in the proportions indicated below to obtain the desired pH:

pH	6.4	6.6	6.8	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	8.0	8.2	8.4
Primary Potas. Phos. c.	73.0	63.0	51.0	37.0	32.0	27.0	23.0	19.0	15.8	13.2	11.0	8.8	5.6	3.2	2.0
Secondary Sodium Phos. c.	27.0	37.0	49.0	63.0	68.0	73.0	77.0	81.0	84.2	86.8	89.0	91.2	94.4	96.8	98.0

Place three c.c. of each of the solutions in suitable small test tubes (100x10 mm., inside measurement). Add five drops of an aqueous 0.01 per cent solution of phenolsulphonphthalein to each tube. Seal off the tops. The series of colors, representing different concentrations of hydrogen-ions, constitutes the standards for comparison of color in carrying out the determination.

Preparation of Sacks.—Dissolve one ounce of collodion (Anthony's negative cotton) in 500 c.c. of a mixture of equal quantities of ether and ethyl alcohol. The solid swells up and dissolves

with occasional gentle shakings, in forty-eight hours. As a small amount of brown sediment separates out at first, the solution should stand for at least three or four days, after which the clear supernatant solution is ready for use. Fill a small test tube (120 by 9 mm., inside measurement) with this mixture, invert, and pour out half the contents. The tube is then righted, and the collodion allowed to fill the lower half again. Invert a second time and rotate on its vertical axis, the collodion being drained off. Care must be taken to rotate the tube, in order to secure a uniform thickness throughout. Clamp the tube in the inverted position and allow to stand for ten minutes, until the odor of ether finally disappears. Fill it five or six times with cold water, or allow it to soak five minutes in cold water. Run a knife blade around the upper rim, so as to loosen the sack from the rim of the test tube, and run a few cubic centimeters of water down between the sack and the glass of the tube. Extract the tube by gentle pulling, after which preserve by complete immersion in water.

The Salt Solution Used in the Method.—Dialyze the blood or serum against an 0.8 per cent sodium chloride solution.

Before applying the test, it is necessary to ascertain that the solution is free from acids other than carbonic. To determine this, place a few cubic centimeters of the salt solution in a Jena test tube and add one or two drops of the indicator, whereupon a yellow color will appear. On boiling, carbon dioxide is expelled, and the solution loses its lemon color and takes on a slightly brownish tint. In the absence of this change, other acids are present, and the salt solution is therefore not suitable. If, on the other hand, on adding the indicator, pink at once appears, the solution is alkaline and hence cannot be used.

Technic of Method.—The technic can be carried out on either serum, plasma, whole, or defibrinated blood. The work must be done in a room free from fumes of acids or ammonia.

Run one to three c.c. of clear serum or of blood, by means of a blunt pointed pipette, into a dialyzing sack which has been washed inside and outside with salt solution and which has been tested for leaks by filling with the salt solution. Lower the sack into a small test tube (100 by 100 mm., inside measurement) containing 3 c.c. of the salt solution, until the fluid on the outside of the sack is as high as on the inside. Allow from five to ten minutes for dialysis.

Remove the collodion sack and add 5 drops of the indicator thoroughly mixed with the dialysate. Then compare the tube with the series of standards until the corresponding color is found, which indicates the hydrogen-ion concentration present in the dialysate.

These tests have been carried out with 3 c.c. of blood or serum. The same results are obtained with 1 c.c. of blood or serum on the inside of the sack and with this amount it is immaterial whether there is 1 or 3 c.c. of salt solution on the outside.

Comparison of Tubes With Standards.—For this, a good light (natural or artificial) and a white background are requisites. Readings must be made immediately. The tube matching most closely is selected and also the tubes on either side of it. These are critically inspected against a white background. Changing the order of the tubes often makes differences more apparent.

Controls of the Method.—Repeated duplicate determinations on the same samples of blood and of serum have convinced Marriott and his co-workers that the limits of error are very slight: for example, the serum from a case of mild acidosis (using quantities of serum varying from 1 to 3 c.c. and dialyzing for from five to fifteen minutes) gave the following series of readings: 7.55, 7.55, 7.55, 7.55, 7.6, 7.55, 7.55, 7.55, 7.55, 7.55. The oxalated whole blood from the same case gave the following readings under similar conditions: 7.25, 7.25, 7.25, 7.25, 7.2, 7.25, 7.25, 7.3, 7.25, 7.25, 7.25; 7.25, 7.25, 7.25.

In order to test out the effect of the variations in the sacks used, a number of determinations were made on the same sample of serum with the following results: ordinary thin sack, 7.7; thick sack, 7.7; opaque, irregular sack, 7.7; ordinary thin sack, 7.65; a very thick sack, 7.7. A series of six normal serums were run through, 3 c.c. and 1 c.c. portions being used for dialysis. In every instance identical readings were obtained.

A brief word of explanation may be given for those unaccustomed to the physicochemical methods of expressing the reaction of a solution. A solution is acid when it contains an excess of hydrogen over hydroxyl-ions, neutral when hydrogen- and hydroxyl-ions are in equal numbers, and alkaline when hydroxyl-ions predominate. An acid of "normal" strength contains, in one liter, a gram of hydrogen capable of forming hydrogen-ions and its strength may be expressed as 1 N. Diluting such a solution ten

times, we would have $1/10$ N or a solution containing $1/10$ gram of actual or potential hydrogen-ions to the liter. Continuing the process of dilution until $1/10,000,000$ normal acid is obtained, we would have in such a solution $1/10,000,000$ gram of hydrogen-ions. Pure water, however, dissociates to form hydrogen- and hydroxyl-ions, and at 20° C. contains approximately $1/10,000,000$ gram of hydrogen-ions to the liter and an equivalent amount of hydroxyl-ions (that is, 17 gm.). That is to say, pure water, our standard of neutrality, is $1/10,000,000$ N acid and also $1/10,000,000$ N alkaline. To avoid writing large figures it is customary to use the logarithmic notation and to express $1/10,000,000$ N as 10^{-7} N or more conveniently, as suggested by Sörensen, to drop the 10 and minus sign and say pH7. If we have less than $1/10,000,000$ gram of hydrogen-ions in one liter the solution is less acid than water, that is, it is alkaline—so, pH8 means actually $1/10,000,000$ N alkali. The higher the exponent, the more the alkaline, or what is saying the same thing, the less acid the solution.

To sum up:

pH1= $N/10$ acid.
 .
 .
 .
 pH6= $N/1,000,000$ acid.
 pH7=NEUTRALITY.
 pH8= $N/1,000,000$ alkali.
 .
 .
 .
 pH14= $N/10$ alkali.

The reaction of the blood serum varies approximately between pH7 and pH8, the neutral point, pH7 being reached only in severe uncompensated acidosis, and a reaction of pH8 being attained perhaps only after administration of alkalies.

The Determination of the Alkali Reserve of the Blood Plasma.

Marriott has recently³ published a method which gives the hydrogen-ion concentration of the dialysate of blood serum after removal of the carbon dioxide, that is in a measure a modification of the indicator analysis of the preceding test, but is more accurate and gives more information than that method. This method serves

³Marriott: Arch. Int. Med., June, 1916, vol. xvii, pp. 840-851.

for the detection and accurate quantitative estimation of the degree of the acidosis.

Apparatus Required.—Set of tubes containing standard phosphate mixtures; a solution of phenolsulphonphthalein in 0.8 per cent. Sodium chloride; collodion sacks; pipette to measure 0.5 c.c.; small test tubes for dialyzing and aerating; atomizer bulb; glass tube or pipette drawn out to a fine capillary point; color comparison box.

Preparation of Phosphate Mixtures—*One-fifteenth Molecular Acid Potassium Phosphate.*—Dissolve 9.078 gms. of the pure recrystallized salt (KH_2PO_4) in freshly distilled water. Add 200 c.c. of 0.01 per cent phenolsulphonphthalein and make up the whole to 1 liter with distilled water.

One-fifteenth Molecular Alkaline Sodium Phosphate.—Expose the pure, recrystallized salt ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) to the air for from ten days to two weeks, protected from dust. Ten molecules of water of crystallization are given off and a salt of the formula $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ is obtained. Dissolve 11.876 gms. of this salt in distilled water. Add 200 c.c. of 0.01 per cent of phenolsulphonphthalein and make up the whole to one liter. The exact amount of indicator is immaterial, provided the same amount of indicator is added to each of the phosphate solutions, and a corresponding amount is added to the salt solution, to be subsequently described. Add a small crystal of thymol to each solution to prevent the growth of molds. The solutions should be preserved in Jena or Non-sol glass vessels. Mix the solutions in the proportions indicated below to obtain the desired pH.

pH	7.0	7.2	7.4	7.6	7.8	8.0	8.2	8.4	8.6
Primary sod. phos., c.c.	37.0	27.0	19.0	13.2	8.8	5.6	3.2	2.0	1.0
Secondary sod. phos., c.c.	63.0	73.0	81.0	86.8	91.2	94.4	96.8	98.0	99.0

Place these solutions in small test tubes, approximately 100 mm. long by 8 mm., internal diameter, of glass that does not readily give off alkali. The tubes are stoppered or sealed off. They should be kept in a dark place when not in use. Under these conditions, the solutions retain their colors for long periods of time.

Preparation of Salt Solution.—Dissolve 8 gms. of chemically

pure sodium chloride in distilled water. Add 220 c.c.⁴ of 0.01 per cent phenolsulphonphthalein solution and make up the whole to one liter with distilled water. The solution should contain no free alkali and no acid other than carbonic. Test the solution by boiling a little of it for a minute or so in a Jena glass test tube, in order to expel carbonic acid.⁵ Cool the solution quickly under the tap and compare with the phosphate standards. Its reaction should be 7.0. If the reaction differs from this, it may be corrected by the addition of a few drops of very dilute acid or alkali to the whole solution. The salt solution must be kept in a vessel of Jena or Non-sol glass, or in a vessel of ordinary glass that has been well paraffined on the inside.

METHOD OF DETERMINATION.—The determination must be carried out in a room free from acid or ammonia fumes. Either serum, oxalated plasma, or blood may be used. Serum is to be preferred, as the addition of oxalate, unless exactly neutral, introduces a source of error. The blood should be collected in a small tube and the serum separated as quickly as possible, preferably by centrifuging.⁶ Hemolysis must be avoided.

Pipette exactly 0.5 c.c. of serum into one of the small collodion sacks, which has previously been washed inside and out with the salt solution.⁷ Lower the sack into a small test tube, approximately 8 mm. internal diameter and 50 mm. long, containing 2 c.c. of the indicator salt solution. The level of the fluid on the outside of the sack should be at least as high as that on the inside. At the end of seven minutes remove the sack and transfer the dialysate to a clean test tube 100 to 140 mm. long and having the same diameter as the tubes containing the phosphate standards. A rapid current of air is bubbled through the solution in order to remove carbon dioxide. This is accomplished by means of an atomizer bulb con-

⁴The concentration of indicator in the salt solution is purposely made 10% greater than in the phosphate mixtures, as during the dialysis a certain amount of indicator is lost by passing into the sack.

⁵If boiled in a soft glass tube, alkali is given off from the glass and the solution is colored pink. Instead of boiling to remove carbon dioxide, the solution may be aerated with a current of air that has been freed from carbon dioxide by passing through a strong solution of sodium hydroxide.

⁶If carbon dioxide escapes from the plasma as a result of shaking or allowing the blood to remain exposed to the air, a passage of alkali from the plasma into the cells occurs with a resultant slight diminution in the alkali reserve of the plasma. Once the plasma or serum is separated from the corpuscles, loss of carbon dioxide is without effect on the alkali reserve.

⁷In washing the sack, no part but the top edge should be touched with the fingers. The sack is emptied by tipping it with a clean, glass rod or with a microscopic slide. Sacks may be used more than once, providing they are thoroughly washed with salt solution after each test.

nected with a narrow glass tube drawn out to a capillary point. The air current should be as rapid as possible without blowing liquid out of the test tube.⁸ Continue blowing for three minutes and then compare the color in the tube with that in the standard phosphate tubes, interpolating when necessary. The reading is a measure of the reserve alkalinity. For convenience of expression this value is referred to as the "RpH" of the serum, to differentiate it from the "pH" as determined in the method previously described by Levy, Rowntree, and Marriott.

RESULTS OBTAINED.—Normal Individuals. The serums of a large number of normal adults were examined by the method described. In every instance the RpH was found to be 8.5 ± 0.05 , provided the subjects examined were on a general mixed diet. A normal adult's serum drawn after a fast of sixteen hours gave a reading of 8.35. The serums of infants gave values slightly lower than those of adults. For normal infants under one year of age, a value of 8.3 for the RpH of the serum was not infrequently encountered. This may be due partly to the fact that infant's blood is usually obtained by cupping; the lower value, however, is more likely an evidence of the tendency towards acidosis that is known to be present in infants.

This accords well with the observed fact that the carbon dioxide tension in the alveolar air of infants is lower than that of adults, and that the combined carbon dioxide of the plasma is less in infants and that the ammonia co-efficient in the urine is often higher. This slight acidosis might well be the result of the more active metabolism of infants, leading to a proportionately greater production of acids.

ACIDOSIS.—A series of cases exhibiting clinical or laboratory evidences of acidosis were studied. The cases included nephritis and diabetes in adults, and nephritis, recurrent and idiopathic acetone-mia, and severe diarrheas in children. The diarrheal cases were of the type described by Howland and Marriott.

In all the cases of acidosis studied, the RpH of the serum showed deviations from the normal. The more severe the acidosis, as indicated clinically or by various laboratory methods, the lower were the figures obtained for the RpH. Especially striking was the

⁸Foaming rarely occurs. It may be present as a result of allowing some serum to spill over the outside of the sack. In case foaming is great enough to be troublesome, it may be prevented by adding a drop of octyl alcohol or toluol.

parallelism between alveolar carbon dioxide tension and the RpH. The two values should correspond, as explained above, provided the respiratory center does not vary in its excitability and the pulmonary epithelium is not damaged in such a way as to prevent equilibrium being attained between the air in the pulmonary alveoli and the blood in the pulmonary capillaries. Thus a hyperexcitable respiratory center should lead to a low alveolar carbon dioxide tension, with a coincident normal alkali reserve. A diminished permeability of the pulmonary epithelium would result in a lowering of carbon dioxide tension in the alveolar air, but not necessarily to a diminution in the alkali reserve of the plasma.

In a number of instances the combined carbon dioxide of the plasma was determined according to the method described by Van Slyke. The results obtained were in a general way proportional to the RpH of the serum. The RpH invariably showed an increase following administration of alkalies, but did not necessarily reach its normal value. It was in connection with the alkali therapy that Marriott found the method of especial value, as it gave information as to the probable amount of alkali needed to replenish the reserve. A determination following the administration of alkali showed whether the amount was sufficient.

Interpretation of Results.—The values obtained for the RpH of the serum may, in the light of his experience, be interpreted as follows:

Values for the RpH of from 8.4 to 8.55 correspond to alveolar carbon dioxide tensions of from 38 to 45 mm., and are to be considered as normal values for adults. Values between 8.0 and 8.3 correspond to alveolar carbon dioxide tensions of from 28 to 35 mm., and indicate a moderate degree of acidosis.

When the value for RpH is as low as 7.7, corresponding to an alveolar carbon dioxide tension of 20 mm., the individual is in imminent danger. During coma, an RpH as low as 7.3 corresponding to an alveolar air of 11 mm., was observed. In infants under one year of age a value for RpH of 8.3, corresponding to 35 mm. tension in the alveolar air, is not to be considered abnormal.

It has been Marriott's experience in general that unless the RpH of the serum is below 7.9, the acidosis may be successfully combated

by dietetic regulation or by the administration of alkali by mouth. When the R_pH of the serum falls below 7.9, intravenous administration of alkali is usually indicated.

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PART II.

CHEMICAL ANALYSIS OF URINE

CHAPTER XIII.

TOTAL NITROGEN.

The method given below is a slight modification of the method given by Myers and Fine which in turn is a modification of the colorimetric method of Folin and Farmer. The only difference in technic is that of adding peroxide of hydrogen to hasten oxidation, which considerably shortens the time of making the test. In the method of Myers and Fine, fully fifteen to twenty minutes is required to complete the determination. As here described, the estimation may be completed in from five to ten minutes.¹

For the determination, an amount of urine sufficient to contain between 0.35 and 0.75 mgms. nitrogen is required. This is usually obtained by a 1 to 25 dilution of urine, although sometimes a dilution of 1 to 10 is sufficient, as indicated by a low specific gravity. Take 1 c.c. of urine with an Ostwald-Folin pipette and dilute to 25 c.c. with distilled water in a volumetric flask. After mixing thoroughly, place 1 c.c. of this material in a thin glass test tube, to which is added 5 to 7 drops (0.1 c.c.) of concentrated sulphuric acid, 50 to 100 mgms. of potassium sulphate, and a drop of copper sulphate (10%). Now boil the tube by hand (or in the apparatus as shown in Fig. 17) with continued shaking (if boiled in apparatus no shaking is required) until the contents become dark brown, and then, while the tube is warm (not hot), add a drop of hydrogen peroxide, and if not clear, heat about one minute until clear. It is this part of the technic that we have modified; namely, the addition of peroxide of hydrogen. When digestion is completed, allow the tube to cool for one minute and then wash into a 50 c.c. volumetric flask or accurate 50

¹Gradwohl and Blaivas: Jour. Am. Med. Assn., Sept. 9, 1916, vol. lxvii, p. 809.

c.c. graduate (A) with about 35 c.c. distilled water. Pipette 5 c.c. of ammonium sulphate solution² containing 1 mgm. of nitrogen per 5 c.c. with an Ostwald-Folin pipette into a 50 c.c. volumetric flask (B), if the Hellige colorimeter is to be employed, and add about 30 c.c. of distilled water. Dilute 10 c.c. of the modified Nessler's solution³ with 40 c.c. of distilled water just previous to use, mix, and make up at once the material in the second volumetric flask (standard) to volume with the diluted Nessler's solution. The flask (A), unknown, is then made up to volume with the diluted Nessler's solution, as in flask B, except that the Nessler's solution is added slowly at first while rotating the flask, until the alkali of the Nessler's solution has neutralized the sulphuric acid. Fill a dry, glass-stoppered wedge for the Hellige colorimeter with the standard solution (see Plate I for the standard color of 1 mgm. of nitrogen) and adjust in the colorimeter. Next place slightly over 2 c.c. of the unknown solution in the empty cup, insert in the colorimeter, and match the colors, preferably with a north light. The amount of nitrogen in 1/25 c.c. of urine may be ascertained in the following table from which the nitrogen content of the specimen of urine under examination may be easily computed.

Since the figures in the table are given for a dilution of 100 c.c., and the dilution here employed is 50 c.c., the result obtained should be divided by 2.

Example.—The twenty-four hour specimen of urine contains 1500 c.c. Our dilution is 1 to 25. Suppose the dilution is 50. Reading is 75, which is equivalent to 0.56 mgm. per dilution of 100 c.c. Divide by 2 equals 0.28 (our dilution is 50); multiply 0.28 by 25 to obtain the amount in 1 c.c. which is 7 mgms., multiplied by 1500 is 10,500 mgms. or 10.5 grams of nitrogen in 1500 c.c. urine.

²This standard solution is prepared by dissolving 0.944 gm. of ammonium sulphate in distilled water and making up to 100 c.c.

³For one liter we need 100 gms. of mercuric iodide, 50 gms. of potassium iodide, and 200 gms. of potassium hydroxide. Place the mercuric iodide and potassium iodide, both finely powdered, into a liter volumetric flask and add about 400 c.c. of distilled water. Now dissolve the potassium hydroxide in 500 c.c. distilled water, cool thoroughly, and add with constant shaking to the mixture in the flask. Then make up to one liter with water. This usually becomes perfectly clear. Keep at 37° C. in incubator over night or until the yellowish white precipitate which may settle out is thoroughly dissolved and only a small amount of the dark brownish precipitate remains. The solution is now ready to be siphoned off and used.

TABLE VI⁴

ESTIMATION OF NITROGEN WITH THE HELIGE COLORIMETER					
COLORI-METRIC READING	NITROGEN MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	NITROGEN MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	NITROGEN MGMS. PER DILUTION OF 100 C.C.
20	1.73	40	1.31	60	0.89
21	1.71	41	1.29	61	0.87
22	1.69	42	1.27	62	0.85
23	1.67	43	1.25	63	0.83
24	1.65	44	1.23	64	0.81
25	1.62	45	1.20	65	0.78
26	1.60	46	1.18	66	0.76
27	1.58	47	1.16	67	0.74
28	1.56	48	1.14	68	0.72
29	1.54	49	1.12	69	0.70
30	1.52	50	1.10	70	0.67
31	1.50	51	1.08	71	0.65
32	1.48	52	1.06	72	0.63
33	1.46	53	1.04	73	0.61
34	1.44	54	1.02	74	0.59
35	1.41	55	0.99	75	0.56
36	1.39	56	0.97	76	0.54
37	1.37	57	0.95	77	0.52
38	1.35	58	0.93	78	0.50
39	1.33	59	0.91	79	0.48

⁴Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

CHAPTER XIV.

UREA.

Dilute the urine 1 to 10 with distilled water. Pipette 2 c.c. of the diluted urine into a test tube of such dimensions that it will easily slip into a 100 c.c. graduated cylinder (no lip), add about 0.1 gm. of urease and incubate the contents in a beaker of water at 50° C. for one-half hour. At the end of this time, add two drops of caprylic alcohol or 1 c. c. of amylie alcohol to prevent foaming in aeration.

We now call attention to the manner of setting up the glassware for the continuation of this test (see Fig. 15). The chemistry of this estimation is about as follows: the enzyme urease converts urea into ammonium carbonate. The ammonia is then liberated by aeration in the presence of sodium carbonate in excess and goes over into the hydrochloric acid as ammonium chloride. This can be determined colorimetrically by the use of Nessler's reagent. There should be two cylinders for each sample of urine. If more than one urine is to be examined, these cylinders may be run in series, two for each test. One cylinder is graduated, the other nongraduated. A two-hole rubber stopper is placed in each cylinder. Cylinder 1 (*A-A'*) is graduated and is connected with the suction. Cylinder 2 (*B-B'*) is nongraduated and is connected with the acid wash bottle (*C*). If more than one urine is under examination, cylinder 2 is connected with the short connection of the other graduated cylinder, etc. This acid wash bottle is simply a bottle containing sulphuric acid (10%) placed at the end of the outfit to prevent ammonia from the air from gaining entrance into the test. Cylinder 1 (*A-A'*) has a short tube bent at right-angles connected to the suction and only extending in the cylinder to a point just within the cylinder. This is tube *F-F'*. Tube *G-G'* extends almost to the bottom of cylinder 1. The end of tube *G-G'* is sealed and a number of small holes are punched in its side with platinum wire which is at white heat, provided the glass is only moderately hot. Cylinder 2 has a right-

angle tube extending to a point just below the stopper (*D*). It has another tube with a straight open end dipping into the test tube (*E*) and running out to be connected either with the acid wash bottle extension or with another series of cylinders in case more than one urine is under examination. Into the 100 c.c. graduated cylinder (cylinder 1) add 20 c.c. distilled water and 2 to 3 drops of 10% hydrochloric acid. This is now closed and cylinder 2 opened. To the test tube containing the digested urine allow an equal volume of saturated sodium carbonate to slowly run down the side of the tube under the urine. Now immediately and carefully insert the tube into cylinder 2 and immediately close, and then carefully and tightly seal the connection. Start the suction slowly by means of the Chapman pump and continue slowly for about five minutes, and then increase the speed of the suction as much as the apparatus will stand. Keep up the aeration for thirty to forty-five minutes. At the end of this time disconnect the cylinders, and cylinder 1 is used for the final determination. Remove the rubber stopper from cylinder 1 and wash down the tube with distilled water (2 to 3 c.c.).

We now come to the development of color. Into a 50 c.c. volumetric flask, pipette 5 c.c. of ammonium sulphate solution¹ containing 1 mgm. of nitrogen, add 25 c.c. distilled water and 20 c.c. Nessler's solution² diluted 1 to 5 (see Plate I for standard color of 1 mgm. of nitrogen). To cylinder 1 containing the unknown in the form of ammonium chloride, add from 10 to 25 c.c. of diluted Nessler's solution (1 to 5), depending upon the depth of color, and dilute to 50 c.c., 100 c.c., etc., depending upon the color. Make the colorimetric reading at once and compare and compute from the table for the estimation of nitrogen with the Hellige colorimeter (see page 78).

The result will be for 0.2 c.c. of urine (urine diluted 1 to 10 for this test and 2 c.c. of diluted urine taken for the determination which is equivalent to 0.2 c.c. urine).

Example.—The twenty-four hour specimens contain 1500 c.c.; dilution is 100; reading is 58. Equivalent from table is 0.93 mgms. in 0.2 c.c. urine. Multiply by 5 equals 4.65 mgms. in 1 c.c. urine;

¹See footnote 2, page 77.

²See footnote 3, page 77.

multiply by 1500 equals 6975 mgms. in 1500 c.c. urine or 6.975 grams Urea N in 1500 c.c. urine.

The amount of urea is computed by multiplying the urea nitrogen by the factor 2.14.

Example.—Urea nitrogen from above equals 6.975 grams, multiplied by 2.14, equals 14.9265 grams of urea in 1500 c.c. urine.

To obtain an accurate figure for the urea nitrogen it is necessary to make a correction for the amount of ammonia nitrogen originally present.

CHAPTER XV.

AMMONIA.

An amount of urine sufficient to give 0.75 to 1.50 mgms. of ammonia nitrogen should be employed. With normal urines 2 c.c. will generally yield the desired amount. With very diluted urines 5 c.c. may be required, while with diabetic urines, rich in ammonium salts, 1 c.c. may be excessive, thus requiring dilution. Pipette the desired amount into a test tube about 200 mm. in length and of sufficient diameter so that it will slip easily into a 100 c.c. graduated cylinder (no lip).

Aeration is carried out in the following manner: To cylinder 1 add 20 c.c. of distilled water and 2 to 3 drops of 10% hydrochloric acid; then close the cylinder and connect cylinder 2 (100 c.c. nongraduated) to cylinder 1 and the acid wash bottle (see Fig. 15). In the test tube containing the urine place 1 c.c. of amylic alcohol or 2 to 3 drops of caprylic alcohol (to prevent foaming), and allow about 3 to 5 c.c. of saturated sodium carbonate to run down the tube gently (under the urine) so that none of the ammonia will escape. Place the test tube in the 100 c.c. cylinder (nongraduated) and then quickly insert the stopper, being careful that the apparatus is properly connected. Start the air from the suction slowly through the apparatus, increasing the speed gradually so that at the end of about 5 minutes the air current is as rapid as the apparatus will stand. Aeration is complete in 15 to 20 minutes. Disconnect the apparatus and use cylinder 1 for the final determination. Remove the rubber stopper from cylinder 1 and wash down the tube with distilled water (2 to 3 c.c.).

We now develop the color. In a 50 c.c. volumetric flask, pipette 5 c.c. of ammonium sulphate solution¹ containing 1 mgm. of nitrogen, add 25 c.c. distilled water and 20 c.c. Nessler's solution² diluted 1 to 5. To cylinder 1 (graduated) containing the un-

¹See footnote 2, page 77.

²See footnote 3, page 77.

known, add 15 to 25 c.c. of diluted Nessler's solution (1 to 5), depending upon the depth of color, and dilute to 50 c.c., 100 c.c., etc., depending upon the depth of color. The colorimetric reading should be made at once. Calculation is made from the table already given (see page 78), and the results recorded as ammonia nitrogen.

Example.—Suppose the twenty-four hour specimen contains 1500 c.c. urine; our dilution is 100, reading 69.

Suppose 2 c.c. were used in the determination. Equivalent from table is 0.70 mgm. in 2 c.c. urine. Divide by 2, equals 0.35 mgm. in 1 c.c. urine; multiply by 1500, equals 525 mgm. in 1500 c.c. urine or 0.525 gram of ammonia N.

CHAPTER XVI.

URIC ACID.

Into a 15 c.c. conical centrifuge tube pipette 2 c.c. of urine, and add 15 drops of ammoniacal-silver-magnesium mixture.¹ Invert the centrifuge tube in order to mix the contents and then place the tube in the refrigerator for about ten minutes, after which centrifuge the tube for from 3 to 5 minutes, and then pour off the supernatant fluid by inverting the tube. (The precipitate will remain at the bottom.) Wipe the lip of the centrifuge tube with filter paper. Volatilize the ammonia by attaching the mouth of the tube to the suction. We are now ready for the development of color, and the reading. As previously mentioned, we must again urge the beginner to work as fast as possible as the color may fade or turbidity may develop.

Prepare a 100 c.c. graduated cylinder for the unknown and a 50 c.c. volumetric flask for the standard solution.² Then pipette 5 c.c. of uric acid standard (5 c.c. equals 1 mgm. of uric acid) into the 50 c.c. volumetric flask. To the standard solution add 2 drops of a 5% solution of potassium cyanide, 2 c.c. of Folin-Macallum³ reagent, 20 c.c. of saturated sodium carbonate, and in one minute, add water to the 50 c.c. mark. (See Plate I for the standard uric acid wedge.) To the precipitate in the centrifuge (which is free from ammonia) add 2 drops of a 5% solution of

¹For the preparation of ammoniacal-silver-magnesium mixture, mix 70 c.c. of 3% silver nitrate solution, 30 c.c. of magnesium mixture, and 100 c.c. of concentrated ammonia. Any turbidity which may develop is removed by filtration. The magnesia mixture alluded to is made as follows: dissolve 35 grams of magnesium sulphate and 70 grams of ammonium chloride in 280 c.c. of distilled water and then add 140 c.c. of concentrated ammonia.

²For the preparation of uric acid standard solution, dissolve 9 grams of pure crystalline hydrogen disodium phosphate and 1 gm. of dihydrogen sodium phosphate in 200 c.c. to 300 c.c. distilled water. Filter and make up to about 500 c.c. with hot distilled water. Pour this warm, clear solution on 200 mgms. of pure, dried uric acid (Kahlbaum) suspended in a few cubic centimeters of water in a liter flask. Agitate until *completely* dissolved, and add at once exactly 1.4 c.c. glacial acetic acid. Make up to one liter, mix and add 5 c.c. chloroform. Five c.c. of this solution is equivalent to 1 mgm. of uric acid. This solution should be freshly prepared every two months. Before weighing out the 200 mgms. of uric acid, it is well to dry the quantity from which the measure is to be made in a drying oven at 100° C. overnight.

³For the preparation of the Folin-Macallum reagent, boil 100 gms. of sodium tungstate, 20 c.c. of concentrated hydrochloric acid, and 30 c.c. of 85% phosphoric acid in 750 c.c. for two hours and then make up to 1000 c.c. with distilled water. In boiling it is well to have a funnel over the flask so as to prevent undue evaporation.

potassium cyanide and shake the tube so as to dissolve the precipitate. Add 2 c.c. of Folin-Macallum reagent. Wash the contents of the centrifuge tube into the 100 c.c. graduate with from 15 to 20 c.c. saturated sodium carbonate. If the color is well developed, more carbonate is used; i. e., use the 20 c.c. amount when the color is stronger than the standard, and the 15 c.c. when it is fainter. The fundamental principle of these dilutions in microchemical work is to have the unknown solution weaker in color than the standard. A space of time of from forty to sixty seconds should be allowed to elapse before determining whether we are going to dilute to 50 c.c. or 100 c.c. Dilute with distilled water to 50 c.c., 100 c.c. depending upon the depth of color obtained. The table for estimation of uric acid with the Hellige colorimeter gives the data for working out the amount of uric acid present. (See page 40 for uric acid table.)

Example 1.—Suppose the volume of urine for 24 hours is 1500 c.c. Dilution is 100 c.c. Reading is 60. Equivalent from table is 0.88 mgm. in 2 c.c. urine. Divide by 2, equals 0.44 mgms. in 1 c.c. urine; multiply by 1500 equals 660 mgms. in 1500 c.c. urine or 0.66 gram in 1500 c.c. Since uric acid contains 33% nitrogen; the amount of uric acid nitrogen may easily be computed from this factor when it is desired.

Example 2.—Uric acid (above) equals 0.66 gram; 33% of 0.66 gram equals 0.2178 gram of uric acid.

CHAPTER XVII.

CREATININE.

Into a 100 c.c. volumetric flask or cylinder, pipette 2 c.c. of urine. Add 3 c.c. of saturated picric acid and 1 c.c. of 10% sodium hydroxide. Mix the solution thoroughly and allow to stand for five minutes. This is done to allow for the development of color. At the end of this time make up the mixture to 100 c.c. with tap water, thoroughly mix and read several times in the colorimeter, using normal bichromate¹ as a standard. The amount of creatinine in 2 c.c. of urine is obtained by ascertaining the value of the colorimetric reading in the Table VII for the estimation of creatinine. If the concentration of creatinine in the urine is not such that the readings from the colorimeter fall within the

TABLE VII²

ESTIMATION OF CREATININE WITH THE HELLIGE COLORIMETER					
COLORI-METRIC READING	CREATININE MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	CREATININE MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	CREATININE MGMS. PER DILUTION OF 100 C.C.
20	2.46	35	2.13	51	1.78
21	2.43	36	2.10	52	1.76
22	2.41	37	2.08	53	1.74
23	2.39	38	2.06	54	1.72
24	2.37	39	2.04	55	1.69
25	2.35	40	2.02	56	1.67
26	2.33	41	1.99	57	1.65
27	2.30	42	1.97	58	1.62
28	2.28	43	1.95	59	1.60
29	2.29	44	1.92	60	1.57
30	2.24	45	1.90	61	1.54
31	2.21	46	1.88	62	1.51
32	2.19	48	1.85	63	1.48
33	2.17	49	1.83	64	1.45
34	2.15	50	1.81	65	1.42

¹Normal bichromate is prepared by dissolving 24.55 grams of potassium bichromate in distilled water, and making up to 500 c.c.

²Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

figures of the table, repeat the test, using larger or smaller amounts of urine as the case may be.

Example 1.—Volume of urine in twenty-four hour specimen is 1500 c.c. Reading on colorimeter is 58. Equivalent on table is 1.62 mgms. in 2 c.c. urine. Divide by 2 equals 0.81 mgms. for 1 c.c.; multiply by 1500, equals 1215 mgms. or 1.215 grams in 1500 c.c. urine.

Creatinine contains 37.2% of nitrogen and if the creatinine nitrogen is desired it may easily be calculated from this factor.

Example 2.—37.2% of 1.215 grams equals 0.45198 gram of creatinine N.

CHAPTER XVIII.

CREATINE.

Place 2 c.c. of urine in a medium-sized test tube and add 2 c.c. of normal hydrochloric acid and a very little powdered metallic lead. Boil the contents of the tube nearly to dryness over a free flame, then wash with as little water as possible through a small cotton or glass wool filter into a 100 c.c. volumetric flask. This removes the metallic lead which also reacts with the picric acid and alkali. To the volumetric flask add 3 c.c. of saturated picric acid and 2 c.c. of 10% sodium hydroxide. Mix the solution thoroughly and allow to stand for five minutes. At the end of this time, make up the mixture to 100 c.c. with tap water, thoroughly mix and read several times in the colorimeter, using the same standard (normal bichromate) and table as for creatinine. The result obtained is the total creatinine. The difference between the preformed and the total creatinine gives the creatine in terms of creatinine. By multiplying this value by 1.16 the weight of the creatine may be obtained.

Example.—Volume of urine in twenty-four hour specimen is 1500 c.c. Reading on colorimeter is 45. Equivalent on table is 1.90 mgms. in 2 c.c. urine. Divide by 2 equals 0.95 mgms. in 1 c.c. urine; multiply by 1500, equals 1.425 grams total creatinine in 1500 c.c., viz.:

Reading = 45.

Table equivalent = $1.90 \div 2 = 0.95$ in 1 c.c. $\times 1500 = 1.425$ grams = total creatinine in 1500 c.c.

Total creatinine 1.425 grams in 1500 c.c. urine (preformed creatinine, 1.215 grams in 1500 c.c. urine) creatine in terms of creatinine equal 0.210 gram in 1500 c.c. urine. Multiply 0.210 by the (above) factor 1.16 which equals 0.2436 gram of creatine in 1500 c.c. of urine.

CHAPTER XIX.

PHENOLSULPHONPHTHALEIN.

The phenolsulphonphthalein test for renal function was devised by Rowntree and Geraghty and depends upon the injection into the tissues of a dyestuff which is eliminated rather rapidly by the normal kidney, and can be estimated quantitatively in the urine. Phenolsulphonphthalein (the dyestuff) is nonirritant to the body either when taken by mouth or when injected into the tissues. It, therefore, does no harm to an already weakened kidney. The patient who is to receive the injection is given 300 to 400 c.c. of water about one-half hour previously, in order to assure a free flow of urine.

Procedure.—Inject 1 c.c. of a solution¹ containing 6 mgms. of phenolsulphonphthalein intramuscularly in the lumbar region (the time of the injection being noted). Allow ten minutes for the beginning of the excretion of the drug. Now collect the urine for two hours, each hour being kept in separate bottles, labelled 1st hour and 2nd hour. In other words, after one hour and ten minutes, the urine is collected in bottle number 1, and in two hours and ten minutes the second specimen of urine is collected in bottle number 2. In patients with obstruction to the flow of urine from the bladder, the retention catheter is stoppered and the urine drawn off at the end of each hour. Other patients may simply be allowed to urinate at hourly periods.

One c.c. ampules (Fig. 28) containing 6 mgms. of the dye can be purchased at any reliable drug concern. Fig. 29 shows a carefully graduated syringe for making this injection.

To bottle number 1, add 10 c.c. of a 10% solution of sodium hydroxide and wash the contents into a 1000 c.c. graduate with tap water. Then dilute to 1000 c.c., 500 c.c., etc., depending upon the amount of dye excreted; i.e., the more dye excreted, the greater

¹This solution is prepared by adding 0.6 grams of phenolsulphonphthalein and 0.84 c.c. of 2/N sodium hydroxide to enough 0.75% sodium chloride solution to make 100 c.c. This gives the monosodium or acid salt which is slightly irritant locally when injected. It is necessary to add 2 to 3 drops more 2/N sodium hydroxide which changes the color to a bordeaux red. This preparation is nonirritant.

the dilution. It is then read in the colorimeter with phenolsulphonphthalein as a standard, and the calculation made from the Table VIII. (See Plate I for the color of the standard phenolsulphonphthalein wedge.)

To bottle number 2 also add 10 c.c. of 10% solution of sodium hydroxide and wash the contents into a 1000 c.c. graduate with

Fig. 28.—Phenolsulphonphthalein ampule.

Fig. 29.—Graduated syringe used for the injection of phenolsulphonphthalein.

tap water. Then dilute to 1000 c.c., 500 c.c., etc., depending upon the amount of dye excreted. Then read in the colorimeter and make the calculation as above. The amount of dye excreted in both hours is added together and recorded.²

²Standard phenolsulphonphthalein is prepared by adding 10 c.c. of 10% sodium hydroxide to exactly 1 c.c. of a solution of phenolsulphonphthalein solution containing 6 mgms. of the dye and making up to exactly one liter.

Example.—First hour dilution was 1000 c.c., reading 56, equivalent on table to 45% excretion first hour. Second hour dilution was 500 c.c., reading 40, equivalent on table to 62%, which is divided by two because the dilution was to 500 c.c. and the table requirement is for a dilution of 1000 c.c. The second hour is 31 per cent. The final report is as follows:

1st hour	45 per cent
2nd hour	31 per cent
Total	76 per cent (normal)

TABLE VIII³

ESTIMATION OF PHENOLSULPHONPHTHALEIN

COLORIMETRIC READING	PHENOLSULPHONPHTHALEIN OUTPUT PER DILUTION OF 1000 C.C.	COLORIMETRIC READING	PHENOLSULPHONPHTHALEIN OUTPUT PER DILUTION OF 1000 C.C.	COLORIMETRIC READING	PHENOLSULPHONPHTHALEIN OUTPUT PER DILUTION OF 1000 C.C.	COLORIMETRIC READING	PHENOLSULPHONPHTHALEIN OUTPUT PER DILUTION OF 1000 C.C.
	Per Cent		Per Cent		Per Cent		Per Cent
10	94	30	73	50	52	70	30
11	93	31	72	51	50	71	29
12	92	32	71	52	49	72	28
13	91	33	70	53	48	73	27
14	90	34	69	54	47	74	26
15	89	35	68	55	46	75	24
16	88	36	67	56	45	76	23
17	87	37	66	57	44	77	22
18	86	38	65	58	43	78	21
19	85	39	64	59	42	79	20
20	84	40	62	60	41	80	19
21	82	41	61	61	40	81	18
22	81	42	60	62	39	82	17
23	80	43	59	63	37	83	16
24	79	44	58	64	36	84	15
25	78	45	57	65	35	85	14
26	77	46	56	66	34	86	12
27	76	47	55	67	33	87	11
28	75	48	54	68	32	88	10
29	74	49	53	69	31	89	9

³Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

Indigo-Carmin Test for Kidney Efficiency.—This is the so-called indigo-carmin test of Folkner and Joseph. This substance

comes in tablet form and is manufactured by Bruckner, Lampe & Company. The tablets are blue and are soluble in water. The solution is injected intramuscularly. These two observers found that the elimination of indigo-carmin begins about eight to ten minutes after its injection. The original method of Folkner and Joseph is to examine the bladder through a cystoscope and observe the first appearance of the blue color in the bladder. This is in other words a method of chromo-cystoscopy. The test was modified by Kapsemar and is perhaps better carried according to his technic: both ureters are catheterized and the time of appearance of the blue color is observed from either kidney in this way. The indigo-carmin is mixed in the following manner: five tablets are boiled in 100 c.c. of distilled water for three to four hours. This is enough material for five injections. Preserve in a well stoppered bottle until ready for use. Inject 20 c.c. for a test, boiling it before the test and injecting it while warm. The injection is made into the relaxed gluteal muscles.

This is a good test as a preliminary measure, but is only useful when positive results are obtained. If the blue color is specifically delayed on either side, the result may be interpreted as an indication of local kidney deficiency. Nevertheless, it must be mentioned that there are numerous cases in which there has been well marked kidney insufficiency and yet the blue color appeared promptly on both sides.

Cryoscopy of Blood and Urine.—It was Koranyi who first opened up the path of cryoscopy in connection with kidney diagnosis. The estimation of the freezing point of human blood was first used. It must be assumed that the freezing point in healthy human blood is a constant factor. Upon this point, Koranyi constructed the following principle: if you have normal kidneys, you have a constant freezing point for blood from such people. Let us assume 0.56°C . as the freezing point of normal human blood. The ease of freezing is in proportion to the number of molecules in the blood. In other words, the more molecules present, the more difficult it is to freeze. In diseases of the kidney we have more molecules in the blood, ergo the freezing point of blood from a patient with diseased kidneys is appreciably lowered. This is theoretically a good rule but there are so many exceptions that it is difficult to use this principle in actual practice. There are some

kidney diseases in which, while the blood ought to be concentrated, there ensues such a rapid thinning out that its freezing point may be normal. The use of blood cryoscopy in the diagnosis of kidney disease has been abandoned by nearly every one excepting perhaps Kuemmel, of Hamburg, who according to our latest information continues to use it. The technic is not difficult but there are many sources of mechanical error in the hands of the unskilled. Of more importance in practical work is the cryoscopy of urine. Cryoscopy is a method of determination of the number of molecules present. If you take the urine from two sides, one healthy and one diseased, you will find on the diseased side a urine with a decreased number of molecules for the reason that the kidney is not functioning as well as it should in conditions of health. It is, therefore, throwing out less material, hence a lessened number of molecules, hence freezing of this urine is not difficult; therefore, the freezing point of urine of this kind is *higher* than urine from a healthy kidney. For instance, if the left kidney has a high freezing point, the right kidney a lower freezing point, then it is the left kidney that is diseased. We express the mean average standard freezing point of urine by the large capital Greek letter Delta. The freezing point of urine varies ordinarily between -1.3° and -2.3° C., the freezing point of water being taken as 0° C. Δ is subject to very wide variations, therefore, its interpretation must be taken up with some discrimination. A copious drinking of water will cause the Δ to have as high a value as -0.2° C. A diet containing much salt and deficient in fluids will lower it to -0.3° C. *Marked* variations are of importance in reading disease of the kidney with cryoscopy findings. A concrete example of the reading of cryoscopy might be given as follows:

Example 1.—

Left Kidney

Clear

$\Delta = -2.46$ C.

Right Kidney

Pus

$\Delta = -1.03$ C.

Diagnosis.—Pyuria with disturbance of the right kidney function.

Example 2.—

Left Kidney

Clear

$\Delta = -2.46$ C.

Right Kidney

Pus

$\Delta = -2.11$

Diagnosis.—Pyuria, with disease of the right kidney, but the difference in the freezing points is so slight that it is not possible to absolutely say that the function of the right kidney is materially disturbed.

Cryoscopy is best carried out by means of the Beckman apparatus. This consists of a heavy battery jar with a metal cover with a circular hole in the center. This jar holds the freezing mixture by means of which the temperature of the urine is lowered and estimated. A large glass tube in the center serves as an air-jacket and is inserted through the central hole. Within this is received the small tube containing the urine to be tested. A thermometer graduated in hundredths of a degree is introduced into the inner tube and is held in place by means of a cork so that the mercury bulb is immersed in the fluid under examination but does not come in contact with the glass surface anywhere. A small stirrer drops into the fluid and is used to stir it while it is being frozen. Another stirrer mixes up the ice and rock salt mixture. Rock salt one part and ice 3 parts, makes a good freezing mixture. Make the test as follows: produce a temperature not lower than 3° C. in the freezing mixture. Introduce the urine to be tested in the small test tube, stir both stirrers so as to equalize the temperature slowly and watch the column of mercury in the thermometer which dips into the urine. This mercury will fall slowly as freezing occurs. You will then observe a sudden jump in the mercury column after it falls. The point that it rises to after this jump is the freezing point.

CHAPTER XX.

CHLORIDES.

Pipette 5 c.c. of urine into a small evaporating dish and add about 20 c.c. distilled water. Precipitate the chlorides by the addition of exactly 10 c.c. of standard silver nitrate solution¹ and add 2 c.c. of the indicator.² Run in from a burette standard ammonium thiocyanate until the first trace of yellow shows throughout the mixture on stirring. By subtracting the number of cubic centimeters required to exactly precipitate the chlorides from ten (silver nitrate added) and multiplying by 0.01, the grams of sodium chloride in 5 c.c. of urine are obtained. From this the total chloride output for the twenty-four hour specimen may be computed. The twenty-four hour specimen contains 1500 c.c. urine.

Example.—6.2 c.c. standard ammonium thiocyanate³ used subtracted from 10 equals 3.8 c.c. of silver nitrate (standard) actually required. Multiply this by 0.01 gram (1 c.c. of standard silver nitrate equals 0.01 gram of sodium chloride), equals 0.038 gram of sodium chlorides in 5 c.c. urine. In 1500 c.c. urine there will then be 300 times 0.038 gram of sodium chloride or 11.4 grams of sodium chloride in 1500 c.c. of urine.

¹For the preparation of the standard silver nitrate solution, dissolve 29.06 grams of silver nitrate in distilled water and make up to one liter with distilled water. Each cubic centimeter of such a solution is equivalent to 0.01 gram of sodium chloride.

²For the preparation of the indicator, dissolve 100 grams of crystalline ferric ammonium sulphate in 100 c.c. of 25 per cent nitric acid.

³For the preparation of standard ammonium thiocyanate, dissolve about 13 grams of ammonium thiocyanate in 800 c.c. distilled water. Titrate this solution against the above standard silver nitrate solution, thus ascertaining the amount of water which must be added to the solution to make it equivalent to the silver nitrate solution.

CHAPTER XXI.

GENERAL ANALYSIS.

Urine.

Volume.—This is easily measured in one liter graduates. The volume of urine excreted by normal individuals is influenced greatly by the diet, particularly by the volume of fluid ingested. The normal figures fall within from 1000 c.c. to 1200 c.c.

Pathological conditions which cause increase in the output of urine, may be enumerated as follows:

1. Diabetes mellitus.
2. Diabetes insipidus.
3. Certain diseases of the nervous system.
4. Contracted kidney.
5. Amyloid degeneration of the kidney.
6. Convalescence from acute diseases.

Many drugs, such as calomel, digitalis, acetates, and salicylates, also cause an increase in the output of urine.

Pathological conditions which cause decrease in output of the urine:

1. Acute nephritis.
2. Diseases of the heart.
3. Diseases of the lungs.
4. Fevers.
5. Diarrhea.
6. Vomiting.

Color.—The color of normal urine varies from a very pale yellow to a reddish yellow. The nature and origin of the chief variations in the urinary color are set forth in tabular form by Halliburton, as shown in Table IX.

Transparency.—Normal urine is ordinarily perfectly clear. On standing a few hours a cloud (nubecula) consisting of mucus threads epithelial cells, etc., forms. After a hearty meal the urine is generally turbid, due to the precipitation of phosphates, and

PLATE III.—URINE COLOR REACTIONS.

1. Showing Indican Reaction.
2. Showing Benzidine Test for Blood.
3. Showing Acetone Reaction.
4. Showing Diacetic Acid Reaction.

TABLE IX.

COLOR.	CAUSE OF COLORATION.	PATHOLOGICAL CONDITIONS.
Nearly colorless	Dilution or diminution of normal pigments	Nervous conditions, hydruria, diabetes insipidus, granular kidney
Dark yellow to brown-red	Increase of normal, or occurrence of pathological pigments, concentrated urine	Acute febrile diseases
Milky	Fat globules Pus corpuscles	Chyluria Purulent diseases of the urinary tract
Orange	Excreted drugs	Santonin, crysophanic acid
Red or reddish	Hemateporphyrin Unchanged hemoglobin Pigments in food (log-wood) matter, bilburies, fuchsin.	Hemorrhages, or hemoglobinuria
Brown to brown-black	Hematin Methemoglobin Melanin Hydrochinol and catechol	Small hemorrhages Methemoglobinuria Melanotic sarcoma Carbolic acid poisoning
Greenish-yellow, greenish-brown approaching black	Bile-pigments	Jaundice
Dirty green or blue	A dark blue scum on surface, with a blue deposit, due to an excess of indigo-forming substances	Cholera, typhus; seen especially when the urine is putrefying
Brown-yellow to red-brown, becoming blood-red upon adding alkalis.	Substances contained in senna, rhubarb, and chelidonium which are introduced into the system	

will disappear on the addition of acetic acid. Permanently turbid urines generally arise from pathological conditions.

Odor.—Normal urine has a faint aromatic odor. On standing a long time all urines are decomposed (undergo alkaline fermenta-

tion) and have a very unpleasant ammoniacal odor. Certain drugs (cubeb, myrtol, copaiba, tolu, saffron, and turpentine) impart a specific odor to urine.

Reaction.—The urine of a normal individual is generally acid to litmus. An animal diet yields an acid urine while a vegetable diet may yield a neutral, or even an alkaline urine. The composition of the food taken is probably the most important factor in determining the reaction of the urine. The reaction also varies considerably according to the time of the day the urine is passed. For instance, for a variable length of time after a meal the urine may be neutral or even alkaline to litmus. This change in reaction is common to perfectly healthy individuals. Normal urine becomes alkaline on standing, owing to the conversion of urea into ammonium carbonate by bacteria.

Specific Gravity and Solids.—The specific gravity of normal urine varies ordinarily between 1.015 and 1.025. It may, however, be as low as 1.003 or as high as 1.040 without necessarily indicating any pathological condition. For instance, following copious water or beer drinking, the specific gravity may become as low as 1.003 or lower. Whereas, on the other hand in cases of excessive perspiration it may rise as high as 1.040 or even higher.

In general (normally and pathologically) the specific gravity is inversely proportional to the volume excreted. In diabetes mellitus, however, we may observe a large volume and a high specific gravity owing to the sugar contained in the urine.

For determining the specific gravity the urinometer commonly is used (Fig. 30). This is sufficiently accurate for clinical purposes. The urinometer is always calibrated for use at a certain temperature. If the specific gravity is taken at any other temperature, correction as given below must be made. In making this correction, one unit of the last order is added for every three degrees above the normal temperature and subtracted for every three degrees below the normal temperature.

Example.—The urinometer is calibrated for 15° C.

The specific gravity of the urine at 18° C. is 1.022.

The true specific gravity at 15° C. would be $1.022 + 0.001 = 1.023$.

Solids.—The amount of solids in 1000 c.c. may roughly be calculated by means of Long's coefficient, which is 2.6. This is ob-

tained by multiplying the last two figures of the specific gravity observed at 25° C. by 2.6.

Example.—The twenty-four hour specimen contains 1500 c.c. Specific gravity is 1016.

(a) $16 \times 2.6 = 41.6$ grams of solid matter in 1000 c.c. urine.

(b) $41.6 \times 1500 = 62.4$ grams of solid matter in 1500 c.c. urine.

Toluene is very satisfactory for preserving urine. This is simply

Fig. 30.—Urinometer.

poured into the specimen so that the urine is overlaid with the toluene.

In certain pathological conditions it is desired to have a separate day and night urine. The urine voided between 8 A. M. and 8 P. M. is taken as the day sample, and that voided between 8 P. M. and 8 A. M. is taken as the night sample.

Glucose.

Qualitative Test for Glucose.—Place about 5 c.c. of Benedict's qualitative solution¹ in a test tube and add 8 to 10 drops (not more) of the urine under examination, and boil the mixture vigorously for a minute and a half. It is allowed to cool spontaneously. In the presence of dextrose, the entire body of the solution will be filled with a precipitate, which may be red, yellow or green in color, depending upon the amount of sugar present. (See Plate IV for color of test for glucose.)

If the amount of glucose is small (under 0.3%) the precipitate forms only on cooling. If the urine contains no sugar, the solution either remains perfectly clear, or shows a faint turbidity that is blue in color and consists of precipitated urates, and should cause no confusion. Even very small quantities of dextrose (0.1%) yield precipitates of surprising bulk with Benedict's reagent.

Benedict's Quantitative Estimation of Glucose.²—The titration method of Benedict which is conceded to be far superior to the older titration methods of Fehling and Purdy, is the method which is chosen. This method gives very excellent results and no special or expensive apparatus is required. It is superior to the Lohnstein fermentation, because the results may be obtained at once (about five minutes). It is also superior to the polariscope method in those instances when levorotatory substances (as β -hydroxybutric acid) are present, thus necessitating a determination both before and after fermentation. Place the urine in a graduated burette, pipette 25 c.c. of the volumetric solution³ into a Jena flask of about 150 c.c. capacity, and add 5 to 10 grams of sodium carbonate and a bit of powdered pumice.

Heat the mixture to boiling on a piece of wire gauze with an asbestos mat and run the urine in rapidly from the burette until a chalky white precipitate begins to form. (See Fig. 31.) Then the

¹Benedict's qualitative solution is composed of 17.3 grams of copper sulphate, 173 grams of sodium citrate and 100 grams of anhydrous sodium carbonate (double the weight of the crystalline salt may be employed), made up to one liter with distilled water. In the preparation of the solution, the copper sulphate should be dissolved separately in about 100 to 150 c.c. of distilled water and then added slowly with constant stirring to a filtered solution (about 800 c.c.) of the other ingredients and finally made up to one liter. This solution is permanent.

²Myers and Fine: *Essentials of Pathological Chemistry*, 1913.

³Benedict's volumetric solution also keeps permanently and is composed of 18.0 grams of copper sulphate, 100 grams of anhydrous or double the quantity of crystallized sodium carbonate, 200 grams of sodium or potassium citrate, 125 grams of potassium sulphocyanate, and 5 c.c. of a 5% solution of potassium ferrocyanide, made up to one liter with distilled water. In preparation, the ingredients are dissolved in the same manner as the qualitative reagent, i. e., the copper should be dissolved separately.

1

2

3

PLATE IV.—BENEDICTS' TEST FOR SUGAR.

1. Green—Showing only a Trace of Sugar.
2. Red—Showing a Large Amount of Sugar.
3. Yellow—Showing a Small Amount of Sugar.

Fig. 31—Showing Benedict's method for the quantitative estimation of sugar.

urine is run in more slowly with continuous boiling, until the last trace of blue color disappears, indicating the end point. Chloroform, if present, should be removed by boiling as it interferes with the reaction. Benedict has found that 25 c.c. of the above copper solution were reduced by exactly 50 mgms. of glucose or 52 mgms. of levulose. Myers and Fine have found that 25 c.c. of the above copper solution were reduced by 54 mgms. of galactose or 67 mgms. of lactose. If a large amount of glucose is present, the urine should be accurately diluted and the test carried out in the same way, the final results being multiplied by the dilution.

Example.—The twenty-four hour specimen of urine contained 2000 c.c.

The amount of urine required to reduce 25 c.c. of Benedict's volumetric solution (50 mgms. glucose) was 10 c.c. Therefore 10 c.c. of urine contains 50 mgms. of glucose. 1 c.c. contains 10 divided into 50 mgms. or 5 mgms. 2000 c.c. contains 10,000 mgms. or 10 grams of glucose.

If the above urine were diluted one-half before examination, the result should be multiplied by 2, or 20 grams of glucose.

Albumin.

Normal urine contains a faint trace of albumin which is too slight to be detected by any ordinary method.

Nitric Acid Ring Test (Heller's Test).—Place 1 c.c. of concentrated nitric acid in a small test tube. By means of a pipette with a rubber bulb on one end, and having a rugged edge on the other, allow an equal amount of urine to run gently down the sides of the tube. The liquid should stratify, and if albumin is present, a white ring of precipitated albumin should appear at the point of juncture. If albumin is present in small amounts, the white ring may not appear until the tube has been allowed to stand for several minutes. If the urine is concentrated a white zone, due to uric acid or urates, may form. This may be differentiated from the albumin ring by diluting the concentrated urine with three or four volumes of water. The experienced worker can easily differentiate between the uric acid ring and the albumin ring, since the uric acid ring has a less sharply-defined upper border, is generally broader than the albumin ring, and is often situated above the

point of contact. Various colored zones due to bile pigments, etc., may also appear, but this should not confuse the worker. After the administration of certain drugs, a white precipitate of resin acids may form at the point of contact and may cause the observer to draw wrong conclusions. This ring (if composed of resin acids) will dissolve in alcohol, whereas the albumin ring will not.

Robert's Test for Albumin.—Into a small test tube introduce 1 c.c. of Robert's reagent.³ By means of a pipette with a rubber bulb on one end, having a rugged edge on the other, allow an equal amount of urine to run gently down the sides of the tube. The

Fig. 32.—Graduated conical centrifuge tube.

liquids should stratify, and if albumin is present a white zone of precipitated albumin should appear at the point of juncture. This test is slightly more sensitive than Heller's test and colored rings do not appear, but if uric acid or urates are present, a white zone may also appear and can be differentiated from albumin by dilution as in Heller's test.

Quantitative Estimation of Protein (Purdy).—Into a 15 c.c. graduated conical centrifuge tube (Fig. 32) place 10 c.c. of clear urine, 3 c.c. of 10% potassium ferrocyanide, and 2 c.c. of 50% acetic acid. Shake the tube and set aside for 10 minutes to allow for the precipitation of the albumin, centrifuge the tube for exact-

³Robert's reagent is prepared by mixing five parts of saturated magnesium sulphate and one part of concentrated nitric acid.

ly three minutes, at 1500 revolutions per minute, in an instrument with a radius, including the tubes, of just $6\frac{3}{4}$ inches. Then take the tube out of the centrifuge and the grams of protein per liter are read off from the following table (see Table X). If the amount of protein is very large, the urine should be accurately diluted.

Example.—Precipitate in centrifuge tube is 1.25 which is equal to 2.6 grams of protein per 1000 c.c. 24 hour specimen contains 1500 c.c. Multiply 2.6 by 1.5, equals 3.9 grams of protein in 1500 c.c.

TABLE X

VOLUME OF PRECIPITATE IN GRADUATED TUBE	DRY WEIGHT OF PROTEIN TO LITER	VOLUME OF PRECIPITATE IN GRADUATED TUBE	DRY WEIGHT OF PROTEIN TO LITER
0.25	0.5	2.75	5.7
0.5	1.0	3.0	6.3
0.75	1.6	3.25	6.8
1.0	2.1	3.50	7.3
1.25	2.6	3.75	7.8
1.5	3.1	4.0	8.3
1.75	3.6	4.25	8.9
2.0	4.2	4.50	9.4
2.25	4.7	4.75	9.9
2.5	5.2	5.0	10.4

Acetone.

To 10 c.c. of urine in a test tube add about one gram of ammonium sulphate, 2 to 3 drops of a freshly prepared 5% solution of sodium nitroprusside, and 2 c.c. of concentrated ammonium hydroxide which may be stratified or poured on the mixture. The presence of acetone is indicated by the slow development of a permanganate color. (See Plate III for acetone color.) The delicacy of this reaction is 1 to 20,000. Pathologically, the elimination of acetone (acetonuria) is said to accompany the following:

1. Diabetes mellitus.
2. Scarlet fever.
3. Typhoid fever.
4. Pneumonia.
5. Nephritis.
6. Phosphorous poisoning.
7. Fasting.

8. Grave anemias.
9. Deranged digestive function.

It also frequently accompanies:

1. Autointoxication.
2. Chloroform anesthesia.
3. Ether anesthesia.

It is believed that the output of acetone arises principally from the breaking down of fatty tissues or fatty food within the organism. The acetone elimination has been shown to increase when the patient is fed an abundance of fat-containing food as well as during fasting. In fasting, the decomposition of fat is increased due to the lack of carbohydrate material and acidosis develops. The same is true with a carbohydrate-free diet.

Diacetic Acid.

Diacetic acid generally is excreted under the same pathological conditions as in acetonuria, diabetes, fevers, etc.

Gerhardt's Test.—To about 5 c.c. of urine in a test tube add ferric chloride solution, drop by drop, until no more precipitate forms. If diacetic acid is present, a violet-red or Bordeaux-red is produced. A variety of drugs or their derivatives will give a positive reaction when present in the urine so that a positive result indicates the possible presence of diacetic acid. If confusion due to drugs is suspected, boil the red solution for 2 to 3 minutes. If the color is due to diacetic acid, it should disappear during boiling and not reappear on cooling. (See Plate III for diacetic acid color.)

Indican.

Normally, 5 to 20 milligrams of indican are eliminated in 24 hours. This amount is greatly increased in conditions of excessive intestinal putrefaction. Of the putrefaction products, the indole, skatole, phenol and paracresol appear in part in the urine as ethereal sulphuric acids, whereas the oxyacids pass unchanged into the urine. The potassium indoxyl sulphate content in the urine is a rough indicator of the extent of the putrefaction within the intestine. The portion of the indole which is excreted in the urine is subjected to a series of changes within the organism and is eliminated as indican.

Obermayer's Test.—Shake about 10 c.c. of faintly acid urine with about 0.1 gram of basic lead acetate, and filter. To the clear filtrate in a test tube add an equal volume of Obermayer's reagent,⁴ and about 3 c.c. to 5 c.c. of chloroform. Place the thumb over the mouth of the tube and shake vigorously. On standing a few minutes the chloroform will settle and it will assume a blue color, if indican is present. (See Plate III for color of indican test.) The intensity of the color will vary with the amount of indigo blue which has been brought into solution by the chloroform. Normally, the chloroform should assume only a faint blue color. In other words, normal urine contains a trace of indican. Qualitatively, the depth of blue color may be taken as indicating the degree of indicanuria, i. e., a deep blue indicates a large amount of indican present.

Phosphates.

The total output of phosphoric acid is extremely variable, but the average excretion as P_2O_5 in 24 hours is about 2.5 grams.

Pathological conditions in which the excretion of phosphates is increased:

1. Diffuse periostitis.
2. Osteomalacia.
3. Rickets.
4. Copious water drinking.

Some investigators claim that the excretion of phosphates is also increased in the following:

1. Early stages of pulmonary tuberculosis.
2. Diseases which are accompanied by an extensive decomposition of nervous tissue.
3. Acute yellow atrophy of the liver.
4. After sleep induced by potassium bromide or chloral hydrate.

Pathological conditions in which the excretion of phosphates is decreased:

1. Acute infectious diseases.
2. Pregnancy (in the period during which the fetal bones are forming).
3. Diseases of the kidney (due to nonelimination).

⁴Obermayer's reagent is prepared by dissolving about 3 grams of ferric chloride in one liter of concentrated hydrochloric acid.

Test for Phosphates.—Place 50 c.c. of urine in a beaker or Erlenmeyer flask, add 5 c.c. of accessory solution,⁵ and heat to the boiling point. A standard solution of uranium nitrate⁶ is then run from a burette into the hot solution (drop by drop) until the precipitate ceases to form. A drop of the mixture brought into contact with a drop of 10% solution of potassium ferrocyanide on a porcelain tablet (Fig. 33) should produce a brownish-red color. If this color does not appear, more standard uranium nitrate solu-

Fig. 33.—Porcelain tablet for the determination of phosphates.

tion should be added, i.e., until the brownish-red color appears. The reading on the burette is taken and is calculated as follows:

Multiply the reading on the burette by 0.005 to obtain the grams of P_2O_5 in 50 c.c. of urine.

Example.—24 hour specimen contains 1500 c.c. urine.

Reading on burette is 10.2.

$10.2 \times 0.005 = 0.051$ gram of P_2O_5 in 50 c.c. urine.

$0.051 \times 30 = 1.53$ grams of P_2O_5 in 1500 c.c. urine.

Bile.

When bile pigments are found in urine it may be regarded as a pathological condition. A urine containing bile is yellowish-green

⁵For the preparation of the accessory solution, dissolve 100 gms. of sodium acetate in about 800 c.c. distilled water, then add 100 c.c. 30% acetic acid to the solution and make up to one liter with distilled water.

⁶For the preparation of uranium nitrate, dissolve 44.8 grams of uranium nitrate in about 900 c.c. of distilled water. Titrate this solution with a standard phosphate solution containing 0.005 gram of P_2O_5 per cubic centimeter. This standard phosphate is prepared by dissolving 14.721 grams of pure air-dry sodium ammonium phosphate ($NaNH_4HPO_4 + 4 H_2O$) in distilled water and making up to one liter. The amount of water to be added to the uranium nitrate solution so that 1 c.c. will be equivalent to 0.005 gram of P_2O_5 can be calculated.

to brown in color and when shaken foams readily, the foam being light yellow in color.

Tests for Bile.—The shaking of the urine and observation of the color of the foam is a valuable test for the presence of bile pigments.

Gmelin's Test.—Place 1 c.c. of concentrated nitric acid in a small test tube. By means of a pipette with a rubber bulb on one end, having a rugged edge on the other, allow an equal amount of urine to run gently down the sides of the tube. The liquid should stratify and if bile is present, various colored rings (green, blue, violet, red, and reddish-yellow) will be noted at the point of contact.

Smith's Test.—Place 1 c.c. of dilute tincture of iodine (1 to 10) in a small test tube. By means of a pipette with a rubber bulb at one end, having a rugged edge at the other, allow an equal part of urine to run gently down the sides of the tube. The liquids should stratify and if bile is present a green ring will be noted at the point of contact.

Blood.

Benzine Test.—To about 3 c.c. of a saturated solution of benzidine in glacial acetic acid add an equal volume of hydrogen peroxide (3%) and 1 or 2 c.c. of the urine to be examined. Shake the tube and in the presence of blood a blue or green color will develop. See Plate III for the color of the blood test. A control should always be made using water instead of urine. This is a very sensitive test.

Guaiaic Test.—Place about 5 c.c. of urine in a test tube and add freshly prepared alcoholic solution of guaiac (1 to 60) until the whole becomes turbid. Then add hydrogen peroxide or old turpentine until a blue color appears (if blood is present). This test gives positive results if old or partly putrefied pus is present, even before turpentine or peroxide of hydrogen is added.

Fresh pus gives positive results upon the addition of hydrogen peroxide.

The above test gives a positive reaction before and after boiling (15 to 20 seconds) if blood is present. Pus does not react after boiling.

Milk, pus, saliva, etc., give positive reactions with the guaiac test, but do not respond after boiling from 15 to 20 seconds.

CHAPTER XXII.

MICROSCOPIC ANALYSIS OF URINARY SEDIMENTS.

The value of the microscopic examination of the urinary sediments of pathological urines is of very great importance from the diagnostic point of view. The sediments may be divided into two classes (a) organized, and (b) unorganized sediments.

Preparation of Sediment.—Pour the urine under examination into a conical centrifuge tube (Fig. 34*B*) and centrifuge (Fig. 34*A*)

Fig. 34*A*.—Centrifuge.

Fig. 34*B*.—Conical centrifuge tube.

for from five to ten minutes. At the end of this time, take the tube out of the centrifuge and introduce a pipette into the bottom of the tube, a finger being placed over the upper opening of the pipette so as not to allow any urine to enter the pipette while it is being placed to the bottom of the tube. When the pipette touches the bottom, the finger is removed and the deposit will flow up into the pipette. Again close the upper end of the pipette and place a drop of the sediment on a clean slide. Then place a cover-glass over

the sediment. In our laboratories we first examine the sediment under the low power, care being taken that a good deal of the light is shut off. Casts are not easily seen in the presence of much light. The sediment is then examined under the high power dry lens. In this way any suspicious elements under the low power may be clearly seen under the high power. When the urine is to be examined for bacteria, etc., the sediments are stained (see following chapter) and examined under the oil-immersion lens.

Organized Sediments.—

1. Casts	{	granular.
		hyaline.
		epithelial.
		blood.
		fatty.
		waxy.
		pus.

2. Cylindroids.
3. Epithelial cells.
4. Leucocytes (pus cells).
5. Erythrocytes.
6. Spermatozoa.
7. Urethral filaments.
8. Tissue debris.
9. Animal parasites.
10. Fibrin.
11. Microorganisms.
12. Foreign substances due to contamination.

CASTS.—Casts are moulds of uriniferous tubules. They vary considerably in size, but nearly always have parallel sides and rounded ends. The finding of casts generally indicates some kidney disorder, especially if accompanied by albumin in the urine.

Granular Casts.—The granular material generally consists of albumin, epithelial cells, fat, or disintegrated erythrocytes or leucocytes. The character of the cast varies according to the size and nature of the granules, i. e., finely granular casts or coarsely granular casts (Figs. 35 *A* and *B*).

Fig. 35A.—Granular casts. (After Hawk.)

Fig. 35B.—Granular casts. (After Peyer.)

Hyaline Casts.—Hyaline casts are pale transparent, homogeneous, and are the most difficult form of renal casts to detect under the microscope. They are common to all kidney disorders (Fig. 36).

Epithelial Casts.—Epithelial casts bear upon their surface epithelial cells and are found in large numbers in acute nephritis (Figs. 37 A and B).

Blood Casts.—The appearance of these casts in the urine denotes acute diffuse nephritis, acute congestion of the kidney, or renal hemorrhage (Fig. 38a).

Fig. 36.—Hyaline casts. (After Hawk.)

Fig. 37*A*.—Epithelial casts. (After Hawk.)

Fig. 37*B*.—Epithelial casts. (After Hawk.)

Fig. 38.—(a) Blood casts (yellow in color); (b) Pus casts. (After Hawk.)

Fig. 39.—Fatty casts. (After Peyer.)

Fatty Casts.—The appearance of these casts denotes fatty degeneration of the kidney and are characteristic of subacute and chronic inflammation of the kidney (Fig. 39).

Waxy Casts.—Waxy casts do not appear in any particular form of nephritis, but are rather common in amyloid disease.

Fig. 40A.—Cylindroids. (After Peyer.)

Fig. 40B.—Cylindroids. (After v. Jaksch.)

Pus Casts.—The surfaces of these casts are covered with pus or leucocytes. Pus casts are rare and indicate renal suppuration (Fig. 38b).

CYLINDROIDS.—Cylindroids are often mistaken for casts but are flat and smaller in diameter than casts. These cylindroids or false

casts may become coated with urates and be mistaken for granular casts. These, however, disappear on warming. Cylindroids have no particular significance because they are found in normal and pathological urine (Figs. 40 *A* and *B*).

Fig. 41.—Erythrocytes.

Fig. 42.—Human spermatozoa. (After Hawk.)

ERYTHROCYTES.—These appear in the urine as the normal biconcave or crenated erythrocyte (Fig. 41).

The pathological conditions in which erythrocytes are found in the urinary sediment, are as follows:

1. Hemorrhage of the kidney.
2. Hemorrhage of the urinary tract.

3. Hemorrhage from congestion.
4. Traumatic hemorrhage.
5. Hemorrhagic diathesis.

SPERMATOZOA.—Spermatozoa may appear after coitus or in the following pathological conditions (Fig. 42):

1. Diseases of the genital organs.
2. Nocturnal emissions.
3. Epileptic and other convulsive attacks.
4. They may or may not be motile. They have an oval body and a long, delicate tail.

URETHRAL FILAMENTS.—These peculiar thread-like bodies may be found in normal urines, and also in the following pathological conditions:

1. Acute gonorrhea.
2. Chronic gonorrhea.
3. Urethrorrhea.

These filaments are generally macroscopical. The first morning urine is best to be examined for filaments.

TISSUE DEBRIS.—The finding of fragments of tissue may sometimes throw some light upon a pathological condition. These tissues may be found in the following pathological conditions:

1. Tubercular affections of the kidney.
2. Tubercular affections of the urinary tract.
3. Tumor of the kidney.
4. Tumor of the urinary tract.

It is necessary, however, to make a histological examination of these tissue fragments before coming to a final conclusion as to their origin.

FIBRIN.—Fibrin clots are occasionally found in the sediments of urines, following hematuria.

FOREIGN SUBSTANCES, DUE TO CONTAMINATION.—Care should be taken that such substances as starch granules, hair, fat, sputum, muscle fibers, particles of food, fibers of silk, wool, linen, etc., are not mistaken for any of the true conditions in urine.

Unorganized Sediments.—

1. Ammonium magnesium phosphate (triple phosphate).
2. Calcium oxalate.
3. Calcium phosphate.

4. Calcium sulphate.
5. Calcium carbonate.
6. Uric acid.
7. Urates.
8. Cystine.
9. Cholesterol.
10. Hippuric acid.
11. Leucine, tyrosine.

Fig. 43.—“Triple Phosphate.” (After Ogden.)

AMMONIUM MAGNESIUM PHOSPHATE (TRIPLE PHOSPHATE).—This compound (Fig. 43) is characteristic when the urine has undergone alkaline fermentation, either before or after being voided, and crystallized in two forms, i. e., prisms and the star-shaped feathery crystals. These crystals may rarely appear in amphoteric or *faintly* acid urines, provided the ammonium salts are present in large enough quantity.

The pathological conditions in which these crystals are frequently abundant, are as follows:

1. Retention of urine in the bladder.
2. Paraplegia.
3. Chronic cystitis.
4. Enlarged prostate.
5. Chronic pyelitis.

CALCIUM OXALATE.—These crystals (Fig. 44) appear in the urinary sediment in at least two forms, i. e., octahedral type and the dumb-bell type. They may be found in acid, neutral or alkaline

Fig. 44.—Calcium oxalate crystals.

urines, but are most frequently found in acid urines. Calcium oxalate crystals are found in normal urines, but are increased in the following pathological conditions:

1. Diabetes mellitus.
2. Organic diseases of the liver.
3. Diseases of the heart.
4. Diseases of the lungs.

These crystals are found in the urine after the ingestion of tomatoes, garlic, rhubarb, oranges, asparagus, etc.

CALCIUM PHOSPHATE (STELLAR PHOSPHATE).—Calcium phosphate (Fig. 45) may occur in the urine in the amorphous, granular or crystalline form and are wedge-shaped and often appear in rosette arrangements. These crystals are sometimes mistaken for sodium urate, but may be distinguished from the latter by dis-

solving them in acetic acid. Acetic acid will readily dissolve the phosphate, whereas the urate is much less soluble.

The pathological conditions in which calcium phosphate crystals are abundant are as follows:

1. Retention of urine in the bladder.
2. Paraplegia.
3. Chronic cystitis.
4. Enlarged prostate.
5. Chronic pyelitis.

Fig. 45.—Calcium phosphate crystals.

CALCIUM SULPHATE.—These crystals (Fig. 46) are very rarely seen and are only found in acid urines. Calcium sulphate crystals appear as long, thin, colorless needles or prisms and may be mistaken for calcium phosphate. They are readily distinguished, however, by the fact that calcium sulphate crystals are readily soluble in acetic acid. These crystals (calcium sulphate) are of practically no clinical importance.

CALCIUM CARBONATE.—Calcium carbonate crystals (Fig. 47) almost always appear in alkaline urine, but may occur in amphoteric or *faintly* acid urine. They very frequently appear in the

Fig. 46.—Calcium sulphate. (After Hensel and Weil.)

Fig. 47.—Calcium carbonate crystals. (After Hawk.)

dumb-bell shape and can be differentiated from calcium oxalate, inasmuch as they dissolve in acetic acid, with the evolution of carbon dioxide gas, while calcium oxalate remains unchanged in acetic acid.

URIC ACID.—Uric acid crystals (Fig. 48) appear in acid urines in the following forms:

1. Wedge-shaped.
2. Dumb-bells.
3. Rhombic prisms.
4. Whetstones.
5. Prismatic rosettes.
6. Irregular or hexagonal plates.

Fig. 48.—Uric acid crystals.

These crystals generally appear in the urine colored brownish-red, although occasionally they can be seen perfectly colorless. The presence of uric acid in the urinary sediment does not necessarily indicate any pathological condition; neither does it mean that the uric acid content of the urine is increased.

The pathological conditions in which uric acid is found in the sediment, are as follows:

1. Gout.

Fig. 49.—Acid sodium urate crystals. (After Hawk.)

Fig. 50.—Ammonium urate crystals. (After Peyer.)

2. Acute febrile conditions.

3. Chronic interstitial nephritis.

URATES.—This may appear as ammonium, calcium, magnesium, potassium, and sodium urate. The calcium, magnesium, potassium, and sodium urates appear in acid urines, while the sediment of ammonium urate appears in neutral, alkaline, or acid urines.

Sodium Urate.—Sodium urate (Fig. 49) may be amorphous or crystalline. When crystalline it appears in sheaves or clusters of colorless needles.

Ammonium Urate generally appears in the burr-like form of the “thorn-apple” (Fig. 50), which appears to be balls with spicules attached.

Fig. 51.—Cholesterol crystals. (After Hawk.)

The pathological conditions in which urates may appear in the urine are somewhat similar to those of uric acid.

CYSTINE.—Cystine is rarely found in urinary sediments and appears in the form of thin, colorless, hexagonal plates. It is insoluble in water, alcohol and acetic acid, and soluble in minerals, hydrochloric acid, alkalies, and especially in ammonia.

CHOLESTEROL.—Cholesterol crystals are very rarely found in urinary sediments and ordinarily crystallize in regular and irregular colorless plates which are transparent (Fig. 51). They

may occasionally be found as a film on the surface of the urine instead of in the sediment.

The pathological conditions in which cholesterol crystals have been found in the urine, are as follows:

1. Cystitis.
2. Pyelitis.
3. Chyluria.
4. Nephritis.

HIPPURIC ACID.—This is very rarely found in urinary sediments. The crystals appear as needles or prisms which are generally pigmented in the manner of uric acid crystals.

Fig. 52.—Hippuric acid crystals.

Hippuric acid crystals (Fig. 52) are more soluble in water and ether than uric acid crystals. These crystals have practically no clinical significance.

LEUCINE AND TYROSINE.—These almost always appear in the urine together. They may be in solution or as a sediment. Leucine crystallizes in characteristic spherical masses and is highly refractive (Fig. 53).

The pathological conditions in which leucine and tyrosine have been found, are as follows:

1. Acute yellow atrophy of the liver.
2. Acute phosphorous poisoning.

3. Cirrhosis of the liver.
4. Severe cases of typhoid fever.
5. Severe cases of smallpox.
6. Leukemia.

Urinary Calculi.—Urinary calculi are solid masses of urinary sediment and are formed in some part of the urinary tract. The smaller calculi, termed sand or gravel, generally arise from the kidney or the pelvic portion of the kidney. The large calculi are generally formed in the bladder. Calculi are divided into two general classes [according to their composition, i. e., simple (made up

Fig. 53.—Crystals of impure leucine. (After Ogden.)

of a single constituent) and compound (made up of two or more constituents)].

URIC ACID AND URATE CALCULI.—These stones are always colored and vary from a pale yellow to a brownish-red.

PHOSPHATIC CALCULI.—These concretions consist principally of “triple phosphate” and other phosphates of the alkaline earths, with very frequent admixtures of urates and oxalates (Hawk).

CALCIUM OXALATE CALCULI.—This is rather difficult to crush

and generally occurs in two forms, the small (hemp seed calculus) and the medium or the large (mulberry calculus).

The following calculi are rarely found:

1. Calcium carbonate (extremely rare).
2. Cystine (rare).
3. Xanthine (more rare than the cystine type).
4. Urostealith (extremely rare).
5. Fibrin (rare).
6. Cholesterol (extremely rare).
7. Indigo (extremely rare—only two cases have been reported).

In examining the urinary calculi chemically, the most valuable data are obtained by examining each of the concentric layers separately. One should saw the calculi through the nucleus and separate the various layers. Enough material may also be obtained by scraping enough powder from each layer to carry out the examination. If the latter is adapted, the layers should not be separated.

Murexide Test.—To a small amount of unknown in a small evaporating dish add 2 to 3 drops of concentrated nitric acid. Evaporate to dryness over a water-bath. If uric acid is present, a red or yellow residue remains which turns purplish red after cooling the dish and adding a drop of very dilute ammonium hydroxide. The color is due to the formation of ammonium purpurate or murexide. If potassium hydroxide is used instead of ammonium hydroxide a purplish violet color due to the production of the potassium salt is obtained. The color disappears upon warming; with certain related bodies (purine bases) the color persists under these conditions.

The following is a scheme proposed by Heller for the chemical examination of urinary calculi and will be found very useful in determining their composition. Reduce the calculus to powder and proceed as follows:

TABLE XI

ON HEATING THE POWDER ON PLATINUM FOIL, IT

DOES NOT BURN		DOES BURN	
The Powder when Treated with HCl		With Flame	
Does not effervesce		The Powder gives the Murexide Test*	
The Powder gently heated, then treated with HCl		The Powder when treated with KOH gives	
The powder when moistened with a little KOH		No noticeable ammonia reaction.	
Effervesces.		Strong ammonia reaction.	
<p>No ammonia, or, at least, only traces of ammonia. Powder dissolves in Acetic Acid or HCl. This solution is precipitated by ammonia (Amorphous).</p> <p>Abundant ammonia. The powder dissolves in Acetic Acid or HCl. This solution gives a crystalline precipitate with ammonia.</p>		Uric Acid.	
Calcium Oxalate.		Ammonium Urate.	
Calcium Carbonate.		Fibrin.	
Urostealth.		Cystine.	
Xanthine.		Triple phosphate	
Bone-earth (Magnesium and Calcium Phosphate).		Triple phosphate (mixed with unknown amount of earthy phosphate).	

*See page 126 for murexide test.

CHAPTER XXIII.

THE STAINING OF BACTERIA IN URINE.

Freshly voided urine from normal persons is free from bacteria, but on standing it becomes loaded with saprophytic organisms. Fungi are prone to develop quickly in diabetic urine. Actinomyces of the genitourinary tract embodies the finding of the actinomyces in the urine. In general aspergillosis, the *Aspergillus fumigatus* appears in the urine. Of the bacteria to be met with in urine in pathological states, we must consider the *Bacillus typhosus* which is found in at least thirty per cent of all cases of typhoid fever. Again we may find the streptococcus, the staphylococcus, the gonococcus, and the glanders bacillus. These are, of course, met with in specific infections. In nephritis of children we are apt to find the streptococcus and the *Bacillus coli communis*. The latter organism is frequently found in the urine from cases of acute cystitis and pyelitis. The *Staphylococcus pyogenes albus* and *aureus* are seen in cases of acute cystitis and, occasionally the *Bacillus pyocyaneus*.

The organism that is possibly the most important one from the standpoint of diagnosis of urinary sediment is the *Bacillus tuberculosis*. Tuberculosis of the genitourinary tract is not an uncommon condition. Thanks to the exceedingly careful work of the modern urologist, this disease is frequently recognized in time to save life, inasmuch as the Great White plague in this locality is, almost strictly speaking, a surgical condition. Prompt diagnosis and prompt extirpation of a tuberculous kidney will often result in a success. The diagnosis of tuberculosis from the urinary sediment is, therefore, extremely important. Whether the specimen represents a catheterized ureteral specimen or a catheterized bladder specimen, it should be treated as follows:

After obtaining the specimen either through a sterile ureteral or sterile urethral catheter, rapidly centrifugalize the urine. Then pour off the supernatant fluid and fill the centrifuge tube with sterile distilled water, shake to wash out the urinary salts which

interfere with staining, and centrifugalize again. This may be repeated, rejecting the supernatant fluid. Spread the sediment upon a clean glass slide by means of a sterile pipette or platinum loop, allow to dry in the air, and then fix by passing through the flame three times. Stain the specimen just as we stain sputum for the *Bacillus tuberculosis*, i. e., steam for three minutes with carbol-fuchsin; then wash off the excess stain with water and decolorize and counterstain with Gabbet's solution. (Gabbet's solution is made by mixing 2 grams of methylene blue with 100 c.c. of 25% sulphuric acid.) Dip the slide but one minute in this solution and rapidly wash off with water, dry, and examine.

If acid-fast organisms are present, it is well to bear in mind that not only the *Bacillus tuberculosis* but also the smegma bacillus is acid-fast. In other words, microscopic finding of an acid-fast bacillus in urine is not positive proof of tuberculosis. We do not believe that the differentiation may be made by means of the microscope alone, even though some advise the expedient of decolorization with alcohol or with acid for a longer time (the smegma bacillus does not resist acid as long as the tubercle bacillus). Rather would we recommend in all cases the use of the guinea pig in making the diagnosis of renal tuberculosis. This test is carried out by inoculating with the urinary sediment, two guinea pigs that are tuberculosis-free, as determined by the tuberculin test,—one intraperitoneally, the other directly in the mass of inguinal glands. They are kept under observation for three weeks. If, during this time, they have not lost weight or developed symptoms, they usually show no tuberculosis. However, in the event that the guinea pigs do not die within this time, they should be kept three weeks longer and then should be anesthetized to death and examined closely for signs of tuberculosis.

In case there is occasion to examine urinary sediment for simple organisms such as staphylococci, etc., we would recommend the following procedure: Treat the sediment as before, washing out the urinary salts with distilled and sterile water. Smear the sediment and dry on slides. Fix in flame and stain for one minute with Roux's blue which we have found to be the best routine stain for bacteria. Roux's blue is made as follows:

Solution A.

Violet dahlia	1 gm.
Absolute alcohol	10 gms.
Distilled water	q.s. for 100 gms.

Solution B.

Methyl green	2 gms.
Absolute alcohol	20 gms.
Distilled water	q.s. 200 gms.

Prepare each solution separately by rubbing up the dye with the alcohol in a mortar and add the water gradually. Let the mixture stand for 24 hours in a bottle. Then mix the two solutions, filter and store in a well-stoppered bottle.

After staining with the above one minute, wash in water, dry, and examine. This makes a beautiful stain for ordinary purposes and in our experience is better than the much used Loeffler stain.

In cases where Gram staining is necessary, for instance, in attempting to differentiate gonococci from Gram-positive organisms, we would recommend the following modification of the usual Gram method. This possesses the advantage of a permanent and reliable primary stain, thereby being superior to the aniline-oil-gentian-violet mixture that must be made up fresh every time it is used. Spread the urinary sediment, dry in the air and fix in the flame.

1. Stain for 30 to 60 seconds with carbol-gentian violet, which is made as follows:

Gentian violet	1 gm.
Carbolic acid crystals	2 gms.
Absolute alcohol	10 c.c.
Distilled water	100 c.c.

Rub up the gentian violet and the alcohol in a glass mortar, add the carbolic acid and mix; add two-thirds of the water, stirring all the time; pour the mixture in a bottle, then rinse out the mortar with the rest of the water and add it to the mixture in the bottle. Leave for 24 hours and filter into a clean glass-stoppered bottle.

2. Blot up the excess of stain (but do not wash), drop two or three large drops of Gram's solution of iodine (iodine 1 gram., potassium iodide 2 grams, distilled water 300 c.c.) on the smear, and allow it to stain 20 to 30 seconds.

3. Wash in water and dry.

4. Pour absolute alcohol over the film a drop at a time until no more violet stain comes away—usually 30 seconds.

5. Wash in water quickly.

6. Counterstain for one minute with an aqueous solution of saffranin.

7. Wash in water, dry and examine. Gram-positive organisms are stained a deep violet and Gram-negative organisms a delicate light pinkish or safranin color.

CHAPTER XXIV.

DESCRIPTION OF THE COLORIMETER.

The methods of blood and urine chemistry already described entail the use of the instrument known as the colorimeter. The two best known instruments are the Duboseq and the Hellige. We have already referred to the fact that the Hellige is the instrument of choice for practical work, owing to its comparative inexpensiveness and also because much smaller quantities of fluids are necessary in using it, thereby saving considerable in standard solutions which take time to make and are also very expensive. Possibly the Duboseq is an instrument of refinement and therefore particularly important in research work, but for the practical laboratory worker the Hellige suffices. So far as other colorimeters now on the market are concerned, the Kuttner-Leitz, Myers, etc., we are inclined to be dubious as to their usefulness in work of this kind. The disadvantages of the former instrument are the use of wedges or tubes containing permanent colors as standards. The Mecca of that success that comes from the greatest accuracy is in the rapid making and mixing of the standard solutions at the same time and under the same conditions as the unknown. Standard solutions made in this way and used in the colorimeter are necessarily the best. The standards for sugar, i. e., picramic acid, and the standard for the functional kidney test of Geraghty and Rowntree, keep some months, but they come within the scope of the above requirements. We would therefore exclude from consideration all colorimeters using wedges and tubes filled with solutions which are not of the same chemical structure and composition as the unknown. So far as the colorimeter of Myers is concerned, it is not to be recommended, owing to the rapid changes that take place in the standard and the unknown in the rather time-consuming process dilution.

The following description and drawings of the Hellige instrument are taken from the treatise by Prof. Autenrieth and Prof.

Koenigsberger, both of Freiburg, published by F. Hellige & Company.

This apparatus is available for color measurements of every kind and consists of a wooden case the back and front of which are in the form of removable slides, as shown in Fig. 54.

The front slide (*V*) is fitted on its outer side with a slit plate, which forms the observation window and behind this on the inner side is a Helmholtz Double Plate (*DP*). The latter is movable and is held between two spring clips (*KL*), from which it can

Fig. 54.—Representation of Hellige colorimeter.

be readily released for the purpose of cleaning. The back (*Sch*) can be moved up and down in a convenient manner by means of the rack and pinion mechanism (*Z*), seen on the right. The back plate has attached to it the most essential part of the colorimeter, which is a hollow glass wedge filled with a standard solution. On the left side the plate is fitted with a scale (*S*) which travels along a pointer (*d*). The open middle portion of the back between the rack and the scale is covered by a ground

glass plate (*M*), which is held in position by a catch (*h*) at the top and may thus be removed at any time without trouble.

Near the top the sliding back is fitted with a wedge holder (*KH*) and at a corresponding point at the bottom of the slide it is fitted with a grooved wooden block (*B*). To adjust the wedges (*K*) in their proper position, the set screw (*a*) which forms part of the wedge holder should in the first instance be turned counter-clockwise, and the fitting with the bracket attachment pressed firmly upwards. The sealed end of the wedge should then be passed through the hole in the bracket attachment and the wedge

Fig. 55.—Representation of Hellige colorimeter.

let down into the fitting and the set screw turned clockwise, so as to clamp the holder firmly. The wedge should always be inserted with its right angle and the rectangular vertical face turned towards the observer.

The small glass trough (*C*) receives the liquid to be tested. It slides into the trough holder (*TH*), whereby it is attached to the left side of the colorimeter. To set the instrument for taking a reading, the back of the colorimeter case together with the wedge should be moved up or down bodily with the aid of the pinion (*T*) and the reading should be taken when the color

intensity due to the thickness of the standard fluid equals that of the solution being tested.

To read the result the scale division indicated by the pointer should be noted, and the corresponding figure read on the ordinates of the calibration curve of the standard wedge; and from the coordinate abscissa the amount of substance contained in a given quantity of fluid, as noted in the curve table, can be determined.

The wedge should always travel in close proximity to the trough, which is generally ensured without difficulty by applying

Fig. 56.—Representation of Hellige colorimeter.

a gentle pressure from the side. There should never be a bright gap between the two fields under comparison, which should merely be separated by a fine line. All glass fittings, such as the double plate, trough, wedge, and ground glass plate should be dry on the outside and carefully freed from particles of dust.

To examine solutions which are so faintly colored as barely to exhibit any tint when viewed in the ordinary trough, such as when determining very small quantities of ammonia with Ness-

ler's reagent, it is necessary to equip the colorimeter with a long trough shown in Fig. 55. The latter is supplied in two forms, either with a drop-in cover (*f*, Fig. 55) or a glass stopper (*g*, Fig. 55). This trough is held in position within horizontal slides, as shown in Fig. 57. To put it in, the ground glass back should be removed, the wedge put in position, and the back pushed into the slide frame. The long trough with its projecting back should be passed through the opening at the back of the colorimeter into the horizontal trough holder referred to. When the long trough

Fig. 57.—Representation of Hellige colorimeter.

is being used the colorimeter requires to be fitted at the back with a light-screening attachment closed at the end by a ground-glass plate so as to encase that part of the trough which projects from the apparatus.

For determining the proportion of iron present in a solution, the apparatus is supplied with a glass stoppered trough, as shown at *e* in Fig. 55, so as to obviate the evaporation of the ether during the observation.

The various troughs may be cleaned by rinsing them out with a little diluted hydrochloric acid, after which they should be rinsed in rotation with water, alcohol, and ether, and finally dried.

For the success of the colorimetric method it is essential that all solutions so tested should be absolutely clear. *All traces of cloudiness or, what is still more objectionable, any precipitate that may be present, should be removed by filtration. The presence of either is liable to falsify completely the adjustment for equality of color intensity.*

To obtain a reliable reading it is best to use diffused daylight, but it should not be too bright. The apparatus should be placed over against a well lighted background, such as a white wall,

Fig. 58.—Optical arrangement of window of colorimeter.

and the eye should be applied to it within the distance of distinct vision, i. e., nearer than ten inches. After a little practice use may be made of artificial light, but in many cases the turning point in the intensities under comparison is not so well marked as when diffuse daylight is used.

To exclude any accidental light, which may interfere with the accuracy of the reading, a screening tube about six inches long can be supplied, if desired, for attachment to the observation window on the front slide, which can for this purpose be fitted with a brass socket.

The instrument described above is adapted for any species of analysis by the method of color comparison, and may within its proper limits be described as a *universal* instrument, since by

a simple interchange of standardized wedges it can be rendered available for any determination that may present itself. It goes without saying that *every species of analysis requires the use of a specially standardized wedge.*

Special sets of standardized wedges are supplied for various purposes; for instance, the analysis of drinking water, rare metals, etc. It is especially important to note that empty wedges with glass stoppers can be supplied, if ordered, so that the calibration of new standards for special colorimetric determinations can be undertaken by the analyst himself.

PART III.

BLOOD FINDINGS AND THEIR INTERPRETATION.

CHAPTER XXV.

BLOOD SUGAR.

What is the significance of the finding of an undue amount of sugar in blood as compared to the finding of an undue amount of sugar in urine? The true condition of the patient so far as carbohydrate metabolism is concerned may better be seen by an estimation of the amount of blood sugar that he will show, rather than by the degree of glycosuria. As a result of the data which have been obtained by following out these microchemical methods, we know that a hyperglycemia may exist without any glycosuria. Again we have glycosuria without hyperglycemia. The appearance of sugar in the urine in cases of diabetes mellitus, it is assumed, is merely a matter of the threshold point, as it were, having been passed. The threshold point, that is, the time when the sugar increase in the blood is accompanied by a pouring out of sugar in the urine, is a matter of debate. Hammann and Hirschmann,¹ at the 1916 meeting of the American Society for the Advancement of Clinical Investigation, reported from a study of 50 cases that if the blood sugar was not above 0.17 per cent, sugar failed to appear in the urine, but that when it reached 0.18 per cent or more, there was a development of glycosuria. Foster,² at the same meeting, found the renal threshold of permeability to lie between 0.149 and 0.164 per cent, basing his observations upon studies made with patients after undergoing ether narcosis.

From our own experience, there seems to be great difficulty in estimating what the normal threshold point is, and it is for

¹Hammann and Hirschmann: Joslin (quoted), *Diabetes Mellitus*, 1916, p. 74.

²Foster, N.B.: *loc. cit.*

this reason that blood sugar determinations are so vital. We have data which show higher concentration of sugar in blood than are noted by the above investigators, but these patients did not show glycosuria. For instance, a very interesting case, which was studied by the authors, gave us a figure considerably higher than that heretofore considered as the threshold point of renal permeability for sugar. It will be noted from a study of the figures shown in the accompanying chart of the case of Mr. H., that this individual, a diabetic for years, when starved for several days, easily became sugar-free so far as his urine was concerned, but his blood sugar remained high, even though no sugar was present in the urine (Benedict's test). It can thus be seen that a rather high degree of hyperglycemia may exist without any glycosuria. This individual believed that the few days' starvation which made him sugar-free also placed him in a state of normal carbohydrate equilibrium. The result of these blood examinations, however, convinced him of the error of his judgment in this respect.

CASE OF MR. H.

BLOOD			URINE*		
Date	Sugar %	CO ₂ Combining Power of Blood Plasma	Sugar	Acetone	Diacetic Acid
7/10/16	0.330	68			
7/14/16			5% or 96 gms. in 24 hr. specimen	Trace	Trace
7/25/16	0.315	85	2.9% or 78 gms. in 24 hr. specimen	Neg.	Neg.
8/16/16	0.216		Neg.	+	+
8/19/16	0.165	53	Neg.	++++	++++

*+ = Small amount; ++++ = Large amount.

A patient may be truly diabetic and may have kidneys relatively impermeable to sugar up to a very high point. Hence, if only

the urine were examined in such a case, the negative findings would not by any means justify us in eliminating the diagnosis of diabetes mellitus. Again, the finding of abundance of sugar in the urine alone does not give us the most intelligent idea of the condition of the diabetic and the amount of starvation and dietetic treatment necessary to rid him of his glycosuria and his hyperglycemia. Ridding a patient with diabetes mellitus of glycosuria does not by any means indicate that he is in a state of carbohydrate tolerance. We must, if possible, reduce his blood sugar to some figure around the normal of 0.08 to 0.12 per cent. If we can make him "sugar-free" so far as the urine is concerned, together with low blood sugar content, then we have the case in a condition where we can have some hope of the performance of ideal normal metabolism.

Again, it must be remembered that the advantage of a blood chemical estimation of sugar can be seen from a survey of the opinions of the authorities as to what constitutes the "normal" for sugar in the urine. Folin³ states that he could demonstrate the presence of sugar in human urine in nearly every one of the hundred persons upon whom he tried out this procedure and adds, "The amount of sugar present in normal human urine is therefore probably much greater than is indicated by the negative findings recorded on the basis of the clinical qualitative tests for sugar in common use." Benedict,⁴ in a personal communication to Joslin, on the other hand, claims that his qualitative test performed according to his later technic will detect glucose in as low a concentration as 0.01 to 0.02 per cent, provided the urine is of low dilution. Joslin⁵ says that these views hardly coincide nor do they coincide with the views of the older investigators who supposed that normal human urine contained as much as 0.5 per cent. Joslin further states⁶ that, "It seems quite impossible to demarcate sharply between normal and pathological urines with reference to the sugar output." It can thus easily be seen that the importance of blood sugar determinations cannot be overlooked. Here we have a doubtful status as to what constitutes a "normal" amount of sugar in the urine; on the

³Folin: *Jour. Biol. Chem.*, 1915, vol. xxii, p. 327.

⁴Benedict: Joslin (quoted), *Diabetes Mellitus*, J. B. Lippincott Company, 1916.

⁵Joslin: loc. cit.

⁶Joslin: loc. cit.

other hand there does not seem to be any doubt as to what is the normal for blood sugar; it lies between 0.08 and 0.12 per cent; anything above this would be termed hyperglycemia and to this figure we would have to turn in the presence of a "doubtful glycosuria."

In our discussion of the etiology of the disease diabetes and the experimental data of later years that have thrown so much light upon this question, we must not forget to note the pioneer work in this field that laid the basis for our present scientific methods. Von Noorden's work on diabetes,⁷ even though his theoretical foundation has been much disputed, did much to intensify the interest in its study. Von Mering and Minkowski, as early as 1890, laid down certain truths about this disease to which the later work of Allen possibly is attributable. Others who did much in this field were Lèpine, Arthaud, Butte, Remond, Hedon, Gley, Thiroloix, Lancereaux, in France; de Dominicis, de Rinzi, Reale, Gaglio, Caparelli, in Italy; Aldehoff, Sandmeyer, Markuse, Weintraub, Seelig, in Germany; V. Harley, in England; and Schabad, in Russia. The work of Minkowski on dogs seemed to crystallize all the previous thoughts and data into a concrete whole. It might be interesting to note that the train of symptoms which follows removal of all or part of the pancreas in dogs is about as follows: polyphagia, polydipsia, hyperglycemia, destruction of albumin, loss of weight, appearance of acetone, diacetic acid, beta-oxybutyric acid, ammonia in the urine, death in coma, with, of course, glycosuria at first quite abundant, later dwindling down as the source is depleted.

It might be well at this point to review some of the facts of normal and abnormal physiological chemistry so far as the source and destiny of sugar in the body is concerned, after which we can more intelligently survey the various classes of conditions grouped as "glycosurias." A glance at the diagrams (Figs. 59, 60, 61) will show how the sugar in the body that is derived principally from the amount of carbohydrates ingested, is utilized under normal conditions. These carbohydrates are principally starches and sugars. The evolution of carbohydrates in the body takes place by the action of intestinal enzymes, converting them into the

⁷von Noorden: *Die Zuckerkrankheit*, Berlin, 1912.

six hexoses or carbon sugars which find their way as such into the portal vein and thence into the liver. In the liver the sugar is formed into glycogen and the excess sweeps out into the blood stream via the hepatic vein as sugar. It is only under exceptional conditions that the glycogen stored in the liver is called upon for more fuel (sugar). Experimentally, of course, it can be shown that this is true by the finding of much more sugar in the portal vein than in the hepatic vein. The liver function is possibly that of a screen, holding back a large part of the sugar and allowing

Fig. 59.—Diagram illustrating normal sugar metabolism. (From Forcheimer: "Therapeutics of Internal Diseases.")

Fig. 60.—Diagram illustrating the nonutilization of sugar in diabetes. (From Forcheimer: "Therapeutics of Internal Diseases.")

the minor part to go on its way peripherally. Of course it must not be forgotten that this sugar in the circulation is not always immediately demonstrable, i. e., it is stored up in muscle as in liver as glycogen. The liver is a veritable reservoir of glycogen. It is claimed that 14 per cent of the weight of the liver is furnished by its glycogen content. Von Noorden very aptly calls the liver a "glycogen reservoir" and the muscles a "glycogen depot." He means by this that while the percentage of glycogen in liver and in muscle by weight is possibly identical, the call for

glycogen or dextrose is first upon the liver and secondly upon the muscles. Another consideration of this interesting fact would be that the union of the glycogen with the liver cells is not near so firm as the union of the muscle cells with their glycogenic visitor. There is another source of sugar, namely, protein. This was disputed for a long time but now proof seems to be undeniable. Protein is transformed into amino-acids such as glycocoll alanine, aspartic, and glutamic acids, and these in turn go over into dextrose. This was originally proved by the experimental fact that animals fed exclusively upon protein and fat store up large amounts of glycogen.

A very elaborate research on this question can be found in the work of Kuelz.⁸ Von Mering and Minkowski,⁹ in their excellent work on experimental diabetes, rather clearly prove the derivation of some of the sugar in the urine from proteins of the food

Fig. 61.—Diagram illustrating excessive formation of sugar through nonretention of glycogen in the liver. (From Forcheimer: "Therapeutics of Internal Diseases.")

and tissues and from fat. For the first few days after removal of the pancreas, it appears probable that the sources of the sugar are proteins and fats of the body. The most important point from the standpoint of the physiologist, however, is the constant relation between the output of nitrogen and sugar, the so-called D:N ratio of experimental diabetes. From the D:N ratio it is safe to conclude that dextrose is partially derived from protein.

A recent and most important work bearing upon this point of the derivation of glucose from protein is that of N. W. Janney,¹⁰ who states that the serious objections open to the data on this

⁸Kuelz: Reported in Pflueger., Arch. f. d. ges. Physiol., 1903, vol. xcvi, p. 1.

⁹Von Mering and Minkowski: Arch. f. d. ges. Physiol., 1904, vol. cvi, p. 160.

¹⁰Janney, N. W.: Arch. Int. Med., Nov. 15, 1916, vol. xviii, No. 5, p. 584.

line of work in the past are based upon the fact that the feeding experiments are not conclusive, inasmuch as it cannot be demonstrated that all the food material is digested and absorbed and that all the glucose arising from this material, and no more, originates from the protein that has been given the subject. It must be remembered, too, that in diabetes mellitus a certain amount of oxidation takes place and that the capacity of the average human diabetic to utilize glucose frequently may undergo considerable daily variation, even when the diet remains the same. It is also possible, states Janney, that the glucose originating from food protein may be in part synthetically used in the formation of various body substances or may be deposited as glycogen. Again it is inadvisable to use fasting diabetics for these experiments because starvation increases the ability of the organism to oxidize glucose. Another and contrary effect of feeding quantities of sugar-forming proteins to diabetics is to lower the tolerance of the organism for glucose. This is very evident from data accumulated experimentally by Mohr. Another disturbing factor in using the human diabetic is the fact that muscular exercise may decrease the glycosuria under some circumstances and increase it under others.¹¹ The difficulty of preventing diabetics from breaking diet is the chief cause of the error in human experiments. Using dogs with extirpation of the pancreas has been attempted, in these experiments, but this is a poor method because extirpation of the pancreas in dogs is followed by severe affections of the digestive system.

With these facts in mind, Janney tried out these experiments in the course of cases of phlorizin diabetes, developing a technic by which the extent of protein conversion into glucose could be followed with considerable accuracy. The details of this technic may be found in his previous publications.¹² Janney mentions a few facts about phlorizin diabetes which has been so well studied of late years by Lusk and others (see page 150 for further particulars on phlorizin). Where phlorizin is given to dogs, diabetes develops, the reserve of carbohydrates in the body is used up, and in the fasting state the glucose appearing in the urine bears a con-

¹¹von Noorden: *Die Zuckerkrankheit*, 1912, ed. 6, p. 100.

¹²Janney, N. W.: *Jour. Biol. Chem.*, 1915, vol. xx, p. 321.

Janney, N. W., and Csonka, F. A.: *ibid.*, vol. xxii, p. 203.

Janney, N. W., and Blatherwick, N. R.: *ibid.*, vol. xxiii, p. 77.

stant relation to the urinary nitrogen, this so-called glucose-nitrogen ratio averaging 3.4 to 1. Glucose administered to such dogs is quantitatively excreted.¹³ Glucose arising from nontoxic ingested substances fails to be stored up but appears in the urine as such. Janney's experimental work shows that it is probable that all the glucose arising from protein fed to phlorizined dogs is excreted in their urine. This demonstrates that the urinary glucose and nitrogen of fasting phlorizined dogs, which quantitatively excrete ingested sugar, bear the same relation to each other as the extra glucose arising from these animals' own protein ingested by other phlorizined dogs does to the nitrogen contained in these proteins. The sugar excreted under these circumstances represents the maximal amount formed from the animals' body proteins.

Janney's work showed that glucose formation from protein is the same in diabetes mellitus as in phlorizin diabetes. He found that isolated proteins yielded large amounts of glucose in metabolism, varying from 48 to 80 per cent according to the protein examined. Contrary to existing opinions, the animal or vegetable origin of proteins bears no relationship to their ability to produce glucose in the animal organism, this function being found to be mainly dependent on the amounts of sugar-yielding amino-acids entering into the constitution of these various proteins. Janney's studies on glucose formation from body proteins demonstrate that body proteins of man and animals yield about 58 per cent of glucose in metabolism. The nitrogen of these proteins bears about the relation of 3.6 to 1 to the glucose formed from them. This definite establishment of the glucose-nitrogen (D:N) ratio is of value in the prognosis of diabetes. Cases showing a high urinary D:N ratio averaging 3.4 to 1, are to be regarded as grave. The lower the ratio, the more favorable the prognosis. As the glucose eliminated by the fasting diabetic is of protein origin, sugar formation from fat does not take place to any great extent in this disease.

Janney also reported the results of glucose formation from protein foods, using the same technic. In von Noorden's food tables for diabetics, glucose formation from protein has not been taken

¹³Ringer, A. I.: Jour. Biol. Chem., 1912, vol. xxii, p. 422.

into account. By adding the amounts of glucose yielded in metabolism by the proteins of a given food to its carbohydrate content, it is possible to ascertain the actual amount of sugar both set free and formed in the metabolism of such foods. Janney also found from experimental studies that the various proprietary protein foods present no advantages over equal amounts of bread when fed to diabetics, as the large amount of protein present leads to the formation of considerable glucose in metabolism.

When we come to the consideration of the possibility of the derivation of dextrose in the body from fat, we have not yet had sufficient experimental or chemical proof. We know that in plant life carbohydrates seem to undergo transformation into fat, still it has not yet been clearly proved in the animal economy. Foster, in his excellent work,¹⁴ calls attention to this point, quoting from analyses of nuts by du Sablon.¹⁵ The figures are parts per 100.

	OIL	GLUCOSE
On July 6, these nuts showed	3	7.6
Aug. 1, " " "	16	2.4
Sept. 1, " " "	59	0
Oct. 4, " " "	62	0

Again we have the example of the germination of seeds with the disappearance of fats and the appearance of carbohydrates. These facts of plant physiological chemistry do not hold good, however, with the animal organism. Fats are split up into glycerol and the fatty acids, but so far there is no proof of their ultimate conversion into sugar. We know now that the increase of fats in the diet of a diabetic does not increase the amount of sugar in the urine. The von Noorden idea on diabetes has been shown to be erroneous, particularly with reference to the fact that sugar in any quantity results from the catabolism of fat.

The ultimate fate of dextrose in the body is *not* clearly and definitely understood. While we have many theories and many experiments, we cannot place our finger firmly and definitely upon the pivotal point of the change of a normal person, say, into a diabetic. As Foster¹⁶ truly says: "At the present time we must confess that we are quite without sufficient data to form any clear conception of the breakdown of the glucose molecule, and it is prob-

¹⁴Foster, N. B.: Diabetes Mellitus, J. B. Lippincott Company, 1915.

¹⁵du Sablon: Compt. rend., 1896, vol. cxxiii, p. 1084.

¹⁶Foster, N.B: Diabetes Mellitus, J. B. Lippincott Company, 1915.

able in the initial step in the destruction of glucose that the essential deviation of the diabetic from the normal becomes manifest. Certainly the diabetic organism is usually able to handle the cleavage products of glucose. The inability to effect the first cleavage might rest in a change in the cell where oxidation is effected or in the absence of an activator. In the light of our knowledge of other vital processes, we must assume the dependence of these changes upon zymases elaborated in one class of cells, perhaps the muscle, and in order to effect their function probably requiring an activator or hormone secreted perhaps by quite remote and different cells.

Joslin¹⁷ states that he considers every patient a diabetic until the contrary is proved, who has sugar in his urine demonstrable by any of the common tests. At this point it must be remembered that glycosuria simply means sugar in the urine in undue quantities. How this may be brought about independent of the disease diabetes mellitus, we shall now consider. Every medical man is familiar with the classic experiment of Claude Bernard,¹⁸ who, as early as 1845, induced glycosuria in rabbits by his *pique* experiment, i. e., the insertion of a steel stylet into the brain of a rabbit. Bernard thrust his stylet into the inferior part of the calamus scriptorius. This glycosuria persisted several hours provided the animals were in a normal state of nutrition. It was completely inhibited if the animal had fasted for a period prior to the experiment, in other words, if its glycogen had been practically released and burned up from its "reservoir" in the liver. The blood sugar, as well as urine sugar, rises in puncture diabetes. Bernard also showed that nerve stimulation had a profound influence in these experiments. The stimulation of the splanchnics by the stylet in the so-called "diabetic center," of course, causes the liberation of the glycogen in liver and its undue appearance in blood, thence into urine. Stimulation of the cut vagi after puncture of the calamus scriptorius caused the following: stimulation of the central stump induced glycosuria; stimulation of the peripheral stump did not. Eckhard¹⁹ showed that division of the vagus and electrical stimulation will

¹⁷Joslin: loc. cit.

¹⁸Bernard, Claude: De l'origine du sucre dans l'économie animale, Paris, 1848; also Leçons sur le diabète et la glycogénèse animale, J. B. Ballière et fils, 1877, p. 576.

¹⁹Eckhard: Beitr. z. Anat. u. Physiol., 1896, vol. iv, p. 4.

cause temporary glycosuria even several days after the nerve is divided. Sugar may also be caused to appear in the urine by cutting the lower cervical or upper thoracic sympathetic ganglia, as shown by Schiff.²⁰

It is also noteworthy that the adrenal bodies are somehow concerned in glycogenolysis and glycosuria. It was Herter²¹ who first showed that painting the pancreas with adrenal extract caused glycosuria. The application of adrenal extracts has a profound influence upon hyperglycemia and glycosuria. We have alluded before to the fact that the liver combination with glycogen is not nearly so firm as the muscle combination, yet the injection of epinephrin into the blood causes the liberation of sugar more quickly from the muscles than from the liver, according to Kutschmer.²² When animals are made glycogen-free by fasting and the use of phlorizin, the use of epinephrin does not produce glycosuria, indicating that this too, like the *pique* of Bernard, is a form of glycogenolysis. It is a fact that *pique* glycosuria does not occur if the adrenals are previously removed, indicating the influence of these bodies upon this experiment. Other agents, that, like epinephrin, act upon the peripheral blood vessels and cause vasoconstriction with raising of blood pressure, produce glycosuria; for instance, barium chloride. This was shown by Neubauer.²³ He showed also that drugs which cause vasodilatation prevent glycosuria due to epinephrin, e.g., pilocarpine and nicotine.

It might be well to mention the fact that not only actual puncture of the calamus scriptorius causes glycosuria transitoria; increase in intracranial pressure or traumatic pathological conditions of other kinds may do so. One of us²⁴ reported an observation of severe and transitory glycosuria in a case of cerebral hemorrhage due to an intraventricular hemorrhage. In this case the glycosuria lasted several days and disappeared, possibly coincidentally with the using up of all the glycogen in the liver and muscles. Autopsy later showed the clot.

²⁰Schiff: Untersuchung über die Zuckerbildung in der Leber u. den Einfluss des Nervensystems auf die Erzeugung des Diabetes, Würzburg, 1859.

²¹Herter: Medical News, 1902.

²²Kutschmer: Arch. f. exper. Path. u. Pharmakol., 1907.

²³Neubauer: Biochem. Ztschr., 1912, vol. xliii.

²⁴Gradwohl, R. B. H.: Philadelphia Med. Jour., April 22, 1899.

It is claimed by Woodyatt²⁵ that various other drugs, such as phosphorus, carbon monoxide, hydrazine, arsenic, etc., may cause glycosuria by causing the increased glycogenolysis alluded to above.

Another interesting form of glycosuria is that caused by the injection of phlorizin, called "phlorizin diabetes." References to this interesting condition can be found in the literature.²⁶

Phlorizin is a glucoside which can be extracted from the bark of apple and cherry trees. In 1886, von Mering established the fact that the administration of this drug to dogs, geese, and rabbits induced glycosuria. If you give a dog 1 gm. of phlorizin per kilo of body weight, in a few hours you will observe at least 10 per cent of urine sugar. The blood sugar will *not* rise. In other forms of diabetes except this variety, you have hyperglycemia. The sugar will persist in the urine as long as you give the phlorizin. All sugar as it is formed in the body goes out in the urine as sugar. It is claimed by some that in this condition the phlorizin ingested simply throws down the barrier of the kidney filter; in other words, that the kidneys are made absolutely and completely permeable to sugar by some alteration in the secretory epithelia. This overflow of sugar from the blood causes a deficit which is supplied by the pouring out of more glycogen from liver and muscles into the circulating blood as sugar, until all is used up. It is for this reason that there is no undue accumulation of sugar in the blood. Here again we wish to allude to von Noorden's ideas, that at this juncture he thought the supply of sugar in phlorizin diabetes was replenished by protein and fatty tissues of the body.

²⁵Woodyatt, R. T.: quoted in: Wells' Chemical Pathology, Philadelphia and London, second edition, 1914, p. 573.

²⁶von Mering: Cong. f. inn. Med., 1886, vol. clxxxv; Ztschr. f. klin. Med., 1889, vols. xiv and xvi.

Moritz and Prausnitz: Ztschr. f. Biol., 1891, vol. xxvii.

Kuelz and Wright: Ztschr. f. Biol., 1890, vol. xxvii.

Cremer and Ritter: Ztschr. f. Biol., 1892, vol. xxviii.

Minkowski: Arch. f. exper. Path. u. Pharmacol., 1893, vol. xxxi.

Zuntz: Verhandl. d. physiol. Gesellsch., Berlin, 1895, 5, vol. vii.

Levene: Jour. Physiol., 1894, vol. xvii, p. 259.

Coolen: Centralb. f. d. Krankh. d. Harn-Sex.-Org., 1895, vol. vi, p. 530.

Pavy: Jour. Physiol., 1896, vol. xx.

Contejean: Compt. rend. Soc. de biol., 1896, vol. xlviii, p. 344.

Markuse: Allg. med. Centr.-Ztg., 1896, No. 49.

Klemperer: Verhandl. d. Ver. f. inn. Med., 1896, vol. v, p. 18.

Lepine: Semaine méd., 1895, p. 383.

Kolish: Wien. klin. Wchnschr., 1897, No. 23.

Lusk, G.: Ztschr. f. Biol., 1898, vol. xxxvi, p. 82.

It is curious that in a very modern and recent publication,²⁷ a writer calls attention to the fact that phlorizin glycosuria is sometimes called "*renal diabetes*" (italics ours) just as some of the older writers spoke of a condition which we shall presently take up, namely, "renal diabetes." But it is our impression that phlorizin diabetes and renal diabetes are in no way related and should not be confused. It is true that in both conditions there is glycosuria but no hyperglycemia, but otherwise there is no pathology known for either. We must sharply distinguish "renal diabetes" from diabetes mellitus, although we have but little pathology on which to base the gross or minute differentiation. Foster²⁸ and Joslin,²⁹ who have written the most recent works on diabetes mellitus, both insist that the future conception of this so-called "renal diabetic" state must rest upon blood chemical analyses. Foster offers the suggestion that these cases of renal diabetes are really cases of beginning diabetes mellitus, but we must confess that the blood data on such cases does not justify this classification. Our conception of a true case of diabetes mellitus is one with definite hyperglycemia and with possibly glycosuria. If, therefore, we meet with a case that shows no hyperglycemia and with definite increase over the normal values of the urine sugar, we must classify this until further notice as a case of renal diabetes. The cases of renal diabetes so-called that occur during the pregnancy period are sufficiently illuminating to bear description. It is well known that in the pregnant state sugar may at times be found in the urine but no increase of blood sugar occurs; besides, there are no signs or symptoms of diabetes mellitus and the occurrence and presence of the sugar in the urine in no way seems to influence for the bad the pregnant status. These women after the puerperium, show no glycosuria, and yet when they become pregnant again, again show glycosuria. They are justly entitled to be called renal diabetics and in no sense "incipient" cases of diabetes mellitus.

In passing, we therefore urge the use of blood analytical chemical methods in seeking more light upon the differential diagnosis of renal diabetes and diabetes mellitus. Joslin, who has

²⁷Monographic Medicine, D. Appleton & Co., N. Y., 1916, vol. iii, p. 788.

²⁸Foster: loc. cit.

²⁹Joslin: loc. cit.

had a very wide experience in handling and studying diabetes mellitus, states that "renal diabetes rarely occurs. The results of the demonstration of the percentage of sugar in the blood of diabetics, which are now being rapidly accumulated will throw light upon this question. Seven cases of my series must be more carefully studied with this in mind. As yet I am not inclined to classify any of these as renal diabetes." In examining the discussion alluded to by Joslin we regret to note that his observation of the blood sugar did not occur on the same day as his observation of the urine sugar; manifestly giving us no basis for watching the ratio of subsidence. He states that "the urine was usually sugar-free at both the time of the first and last blood tests. It will be of interest to compare these figures with those observed with a subsequent series of patients. It seems remarkable that so many patients should become sugar-free and yet the blood sugar remain so high. Presumably this is due to the short period of time intervening between the first and the last blood test. It would seem to indicate that rigorous dietetic treatment should be continued even for a long period of time after the patient becomes sugar-free."

A very interesting contribution to the literature of renal diabetes is a recent article by Lewis and Mosenthal.³⁰ They state that in this condition the blood sugar does not vary from the bounds of the normal, an increase or decrease in the carbohydrate diet has little effect on the percentage of sugar in the blood or the quantity excreted in the urine. These cases have none of the clinical manifestations of diabetes mellitus, due either to diminished ability of the body to utilize glucose or the presence of a hyperglycemia; there is no polydipsia, polyphagia, or polyuria, no loss of weight or weakness, no pruritus or furunculosis, nor any other symptom of this disease. It remains stationary, the glycosuria shows no tendency to increase, nor does diabetes mellitus develop from it; the subject continues in good health and without any abnormal symptoms except a constant low grade glycosuria. The data necessary for the diagnosis of renal diabetes are very few in number, but sharply defined:

1. A glycosuria, maintained at a fairly constant level and

³⁰Lewis and Mosenthal: Bull. Johns Hopkins Hosp., 1916, vol. xxvii, No. 303, p. 133.

not markedly affected by changes in the carbohydrate content of the food.

2. A normal percentage of blood sugar while the urine contains glucose.

Cases in the literature are not very common. Von Noorden was somewhat skeptical, but Allen³¹ admits two cases, those of Bonniger and Tachau, as absolute examples of the condition. Other cases are those by Graham³² and de Langen.³³ Lewis and Mosenthal's case report is another undoubted case added to the literature. The full history of this interesting case is as follows:

W. P. W., Medical History No. 34774, male, white, age 29, born in the U. S., a station agent, descended from Anglo-Saxon ancestry.

Family History.—Father (aged 60), mother (aged 50), one brother and four sisters are all alive and in good health; one sister died of erysipelas. With the exception of marked obesity in one grandmother and several of her sisters, there is no history of hereditary disease; diabetes mellitus, heart trouble, kidney disease, apoplexy, gout, exophthalmic goiter, and tuberculosis have never been found in the patient's family.

Habits.—Smokes five to six pipes a day; does not use alcohol; eats a considerable amount of bread but no excess of sweets.

Past History.—Measles and whooping cough in childhood, malaria 18 years ago, pneumonia 17 years ago, varicella, complicated by otitis media on the right side, 15 years ago. Venereal infection is denied.

Present History.—Three years ago passed a life insurance examination. This is the only urinary test remembered, until six weeks ago, when the patient applied to his physician for relief from backache. At that time a glycosuria was demonstrated. The backache cleared up shortly; the glycosuria persisted in spite of a restriction of the carbohydrates in food. There never have been any other symptoms pointing to diabetes mellitus with the exception of transient paresthesia of the fingers (no loss of weight or strength, no polyuria, polyphagia, no skin involvement—pruritus, furunculosis or other condition—no muscular cramps, no pains in the extremities); there have been no evidences of pancreatic disease (no pain in the epigastrium, no fatty diarrhea); all indications of exophthalmic goiter have been completely lacking at all times (no exophthalmos, no thyroid enlargement, no vomiting, nervousness, cardiac palpitation, or diarrhea); there have been no signs of acromegaly or gigantism pointing to a hypophyseal involvement; there has been no history of a renal lesion (no headache, visual disturbance, dyspnea, vertigo, edema or albuminuria); there has never been any skin pigmentation to suggest a cirrhosis of the pancreas and liver, that is hemachromatosis.

For the last two or three years there has been a tendency to increased frequency of urination during the day but not at night. The quantities voided have apparently not exceeded normal. This is evidently a pollakiuria

³¹Allen: Glycosuria and Diabetes, Boston, 1913.

³²Graham: Jour. Physiol., 1915, vol. xlix, p. 46 (proceedings).

³³de Langen: Berl. klin. Wchnschr., 1914, vol. li, p. 1792.

rather than a polyuria, which is borne out by the ward observations which will be detailed further on.

There has been a slight chronic cough associated with a moderate nasal catarrh, and mouth breathing. There have been no night sweats, hemoptysis, or "pleuritic pain."

Present Complaint.—The patient feels perfectly well and would not believe himself sick were it not for the persistent, "sugar-in-the-urine."

Physical Examination.—Height 5 feet, 9 $\frac{3}{4}$ inches, weight 152 pounds; appears to be in the best of health and spirits; the skin and mucous membranes are not pigmented, their color is normal, they are as moist as those of a normal individual. The pupils are equal and react to light and accommodation; Von Graefe's sign is absent. The pharynx is injected and there is a moderate degree of nasal obstruction, as indicated by persistent mouth breathing. The tonsils are not enlarged or inflamed. There is no pyorrhea alveolaris. The thyroid is barely palpable. The pulse rate averages 75; the pulse is regular in force and frequency and of normal value. The radial artery can be rolled under the palpating finger, but is soft and elastic. The temperature is normal. The respiratory rate ranges from 16 to 24. The systolic blood pressure is 140, the diastolic 80. The heart's apex beat cannot be seen, it is barely palpable in the fifth interspace, 10 cm. to the left of the median line; the character of the apex impulse is a normal one; there are no thrills over the precordium; the area of relative cardiac dullness extends 3.5 cm. to the right of the mid-line in the fourth space, and 10.5 cm. to the left in the fifth; the heart sounds reveal no murmurs, the second sound over the aortic area is somewhat intensified and is louder than the pulmonic second sound. The lungs are normal except for slight dullness and somewhat prolonged expiration in the right supraspinous fossa, and at times a few dry râles, after coughing, over the same area. The liver and spleen are not palpable and there are no areas of tenderness or increased resistance over the abdomen. The patellar reflexes are very active. There is no edema of the face, back or extremities. On the left thigh there is a small eczematous patch furrowed by scratch marks. The superficial lymph nodes are not enlarged. The hemoglobin is 100 per cent (Sahli), the red blood cells are 4,000,000 and the white blood cells are 8450 per c.mm. The Wassermann test is negative. The urine on admission is clear, of reddish yellow color, specific gravity 1035, acid in reaction, negative for albumin, gives a distinct reaction for sugar, and on microscopic examination yields no casts or red blood cells; the qualitative tests for acetone and diacetic acid are negative; the 'phthalein test shows an excretion of 42 per cent in two hours; Ambard's constant determined at various times is 0.07, 0.11, 0.08, 0.10.

Impression.—The presence of glycosuria was well established. The urine gave a positive reaction with both the quantitative and qualitative Fehling-Benedict reagent, yielded gas on fermentation with yeast, and the unfermented urine rotated the polariscope to the right. The nature of the glycosuria will be subsequently discussed. There may have been a healed tubercular lesion at the right apex; impaired resonance, slightly prolonged expiration and inconstant râles in this region are not pathognomonic of a tuberculous focus; it is certain that in absence of fever, sputum, night sweats, chills and loss of weight an active process is not probable and therefore of no significance in explaining the glycosuria. Of equally little importance is the nasal obstruction and pharyngitis. The kidneys are anatomically intact as

far as the physical and urinary signs are concerned; the functional tests of these organs, however, reveal some impairment as shown by a slightly diminished phthalein excretion and an Ambard's constant barely within what has been in our experience the upper normal figure. The connection between such a diminished kidney function and a possible renal diabetes is of extreme interest. The small eczematous patch in this case could not be regarded as a complication of diabetes mellitus, since the hyperglycemia, which is the direct etiological factor of such a condition, was lacking.

The urinary nitrogen was determined by the Kjeldahl process, the ammonia according to Folin, the glucose by Benedict's modification of Fehling's method, the acid bodies by Shaffer's procedure. The method of Lewis and Benedict was used in estimating the blood sugar.

Blood sugar determined by the Lewis and Benedict method was normal, although urine showed glucose. This case must be classed as one of true renal diabetes. There was slightly diminished phenolsulphonphthalein excretion, the slight elevation of Ambard's constant above the normal, as well as the glycosuria, point to a depressed kidney function. The absence of any further subjective or objective signs, past or present, leads to the conclusion that a renal glycosuria is an interesting anomaly, but of no importance to the organism as a whole.

The question of prognosis in this condition is the most important problem which remains to be solved. It is well known that instances of true diabetes may persist for years without changing from a mild to a severe type in spite of the lack of any systematic efforts at dietary restriction, thus resembling renal glycosuria. It is not certain that what is termed renal diabetes may not develop into diabetes mellitus, especially since comparatively little is known of the early stages of true diabetes. The number of cases of renal glycosuria thus far observed has been small and none of them has been followed for a sufficient length of time to ascertain whether renal diabetes is congenital, and not an acquired anomaly, and whether it may persist indefinitely without changing its characteristics.

The intensity of renal glycosuria should vary with the degree of kidney permeability to dextrose. With a threshold only slightly depressed, an intermittent glycosuria often of an apparently unexplained origin may be present; with a very marked depression, changes approximating the conditions found in phlorizin poisoning should develop. Intermediary degrees of kidney involvement should have glycosuria of corresponding intensity. If the present ideas of the relations of a diminished kidney threshold for sugar are true, all the grades of intensity indicated should be demonstrated in the course of time.

This expresses a very conservative estimate of the facts at hand, that we are not as yet justified in classifying these cases as incipient cases of diabetes mellitus. We have evidence furnished by blood chemical analyses that there is no hyperglycemia in these cases. Until data are at hand showing conclusively that these cases without increased blood sugar but with glycosuria do inevitably lapse into hyperglycemia with the concomitant symptoms of diabetes mellitus, we should not group them in any way

with that disease. Blood chemical methods indeed alone will furnish the evidence which will eventually place these cases in their proper position.

Before passing further into the question of true diabetes mellitus, we might say a word regarding the so-called *alimentary glycosuria*. One formerly distinguished between a form due to the ingestion of starch and that due to the ingestion of sugar (alimentary glycosuria *e saccharo*). Naunyn³⁴ attempted to distinguish an alimentary glycosuria, i. e., one due entirely to the ingestion of carbohydrates, from a case of diabetes mellitus, by a renal test meal. Referring to this question, the *Journal of the American Medical Association*³⁵ states in part:

"In certain individuals the capacity of utilizing glucose is supposed to be lowered. It may become sufficiently deficient in some instances to lead to so-called alimentary glycosuria following an overindulgence in carbohydrate food. In a healthy person it is scarcely possible to produce glycosuria by the lavish administration of starchy food, since the liver can apparently store up the excess of sugar as fast as it is produced by the digestion of starch in the alimentary canal and absorbed into the portal circulation. There is a widespread belief that when preformed glucose is fed, however, the assimilation limit may be more readily reached through rapid and unduly large absorption of soluble carbohydrate. It may become very important to ascertain an incipient functional defect of this sort, since it may be the indication of some impending diabetic defect. Accordingly it has been customary in some clinical laboratories to ascertain the "assimilation limit" for glucose by feeding a measured quantity of this carbohydrate or some other sugar, such as lactose (milk sugar) or levulose (fruit sugar), at one time, and watching for a transient glycosuria as a result. To the examination of the urine for sugar before and after the administration of the carbohydrate, the analysis of the sugar content of the blood may now easily be added.

"Success in ascertaining an abnormal tolerance in a procedure of the sort described evidently hinges on the ability to postulate what a normal functional capacity of a healthy individual in such

³⁴Naunyn: *Der Diabetes Mellitus*, Wien, 1906.

³⁵Editorial: *Jour. Am. Med. Assn.*, Sept. 2, 1916, p. 748.

circumstances should be. Lately it has been asserted that whereas the 'assimilation limit' is low in diabetes, it is abnormally high in certain conditions involving a malfunction of some of the endocrine glands notably the pituitary. Taylor and Hulton,³⁶ of the Department of Physiological Chemistry at the University of Pennsylvania, recently remarked that by common consent, rather than by accurate experimentation, the limit of assimilation of glucose on alimentary administration has been set at from 200 to 250 gms. on the empty stomach. From this figure downward the student of diabetes applies the test; from this figure upward the student of the diseases of the ductless glands applies the test. The Philadelphia investigators have made a number of observations on healthy medical students, to whom glucose was administered in strong solution and in whom blood sugar content was ascertained immediately before and three hours after the sugar was given. As a result it is clear that nearly all the subjects tolerated the ingestion of 200 gms. without exhibition of glycosuria. Of nine subjects who ingested 300 gms., only three displayed glycosuria. Of the six who ingested 400 gms., only two had glycosuria. In five instances 500 gms. were given, with the production of glycosuria in but one. Taylor and Hulton regard 500 gms. as the physical limit of ingestion, except in one who has trained to the test; it is very large in bulk, inclines to nauseate, and apparently the excess is not rapidly absorbed, so that the test probably means no more than does the administration of 400 gms., which is usually tolerated. Polyuria occurred rarely, and there was no relationship between the polyuria and glycosuria. Intestinal disturbances were not observed. It appears, by way of contrast, that healthy persons cannot ingest 300 gms. of levulose without intestinal disturbances. Whether this result is inherent in such amounts of levulose, or is due to some impurity in the supposedly pure preparation used, could not be determined. The further general conclusion was drawn that even the larger quantities of sugar do not markedly influence the sugar content of the blood. In the majority of healthy adult males, according to Taylor and Hulton, there is, apparently, no limit of assimila-

³⁶Taylor and Hulton: Jour. Biol. Chem., 1916, vol. xxv, p. 173.

tion of glucose; a glycosuria does not regularly follow the largest possible ingestions of pure glucose.

"Woodyatt, Sansum, and Wilder³⁷ have very properly pointed out that the common clinical practice of estimating sugar tolerance as the number of grams of glucose which can be given by mouth all at once and just fail to cause glycosuria will not justify any tenable conclusion respecting the power to utilize glucose. They say:

" 'When sugars are administered by the stomach, the length of time during which they are actually brought to the cells must depend on the motor power of the stomach and of the bowel and on the rates at which the sugars can be absorbed; and even when they are given subcutaneously or by any other route which involves absorption as a prelude to their entering the blood, the rates at which they enter the blood will depend on the rates at which they are absorbed. By any of these, but especially by the oral method, the actual rate of entry of sugar into the blood and tissues at large must vary with a wide range of physical, physiologic and pathologic conditions over which we have no control; nor will it ever be possible by such methods to force sugar to enter the blood any faster than it can be absorbed. The rate of sugar absorption is a self-limited thing, for when a certain concentration of sugar is once present in the blood, no quantity given by mouth or subcutaneously or intraperitoneally can raise it higher.'

"The fact that prolonged hyperglycemia did not arise in Taylor and Hulton's trials on normal persons is in itself an indication that one could scarcely expect marked glycosuria to manifest itself. It has been found that a man weighing 70 kgs., when resting quietly in bed, may receive and utilize 63 gms. of glucose by vein per hour without glycosuria. The normal tolerance limit for glucose, expressed as a velocity, is established at close to 0.85 gm. of glucose per kilogram of body weight hourly, which agrees approximately with what Blumenthal has established by repeated small intravenous injections in animals. It can easily be computed from such statistics that if a man's resting requirements were 3,000 calories per day, he could thus receive double what

³⁷Woodyatt, Sansum, and Wilder: Jour. Am. Med. Assn., 1915, vol. lxx, p. 2067.

he needed, or enough to cover the caloric expenditure of the same man during the heavy physical exertion. In view of these facts perhaps the supposed increased 'tolerance' for glucose in some of the ductless gland disorders relates to a gastrointestinal rather than a metabolic function."

The study of diabetes mellitus is attracting great attention at the present time, mainly because of the advent of the Allen starvation treatment. This is based on the results of exact animal experimentation. It is bearing the richest fruit in the form of excellent therapeutic results. Diabetes mellitus is said to be rapidly increasing in incidence, yet this may simply mean that more cases are discovered now that routine urine analyses are being made. Joslin states that the frequency of diabetes in the United States is *one per cent of all individuals* (they either have the disease or will develop it); also that the frequency of diabetes in a community may be the index of the intelligence of its physicians. The routine examination of the urine of every patient should be made the order of the day, not altogether because we want to discover diabetes, but because we want to know something about other conditions. We urge that the Benedict test for sugar be given the preference over all other sugar tests of urine. It is made from a solution that is stable, and besides, shows sugar at times when Fehling's test does not. This has occurred in our experience a number of times. The routine examination of urine does not mean the examination of the single specimen in the morning before breakfast. It may be surprising to some to learn that at this time sugar is often absent from the urine of a diabetic.

If one must rely on urinary tests and not utilize the blood chemical methods, it must be remembered that there are individuals with a lowered power of assimilating carbohydrates who secrete glucose only for short periods in the day, some time after meals, and then only in small quantities. Even true diabetics in the mild stage are often, even apart from diet, free from glycosuria for some part of the twenty-four hours, especially in the morning before the first meal. Kleen³⁸ stated this well known fact as follows: "The first and most important rule is, therefore, never to

³⁸Kleen: Diabetes Mellitus, P. Blakiston's Son & Co., 1900.

use for a test a single specimen of urine passed when the patient's stomach is empty, before the first meal of the day. The best means of deciding from a single examination of the urine whether a person is normal or not in this respect is furnished by a sample passed an hour after the end of the dinner. At this time the excretion is at its maximum."

The routine examination of blood chemically will some day be required in making clinical diagnosis. To recommend this at the present time seems Utopian, yet the results of such a study would certainly repay one who follows it out. The methods which have been described promise accuracy and ease of performance to those qualified to undertake this work. It is true that the advantage of the Allen treatment lies in the fact that the dietetic regime may be carried out without elaborate tests of blood and urine, yet a far better control of the treatment is within our grasp if we resort to blood chemical estimation.

The author's data on the following two cases, blood and urine of which they carefully studied, will demonstrate the discrepancies between the findings in urine and blood of diabetics. The first case, Mrs. R., was under observation twenty-four days, during which time she was given the Allen treatment. This was a young woman of twenty-three, with a history of one brother dying of diabetes. She had developed diabetes mellitus one year before coming under our observation. During this time she had been under various dietetic regulations but had not been able to accomplish much in the way of permanently relieving herself of diabetic symptoms or of glycosuria. She displayed some loss of weight and polyuria and polydipsia. At the time of the first examination she showed 0.360% blood sugar and was excreting 78 gms. of sugar in the twenty-four hour specimen of urine. She had a carbon dioxide combining power of 68, with a large amount of acetone and diacetic acid in the urine. She was watched one week before beginning the Allen treatment, on general diet. During this time she was given 1/10 grain parathyroid three times daily for certain experimental purposes. During this week's observation, she showed a marked increase in the amount of sugar in the urine, but the amount of blood sugar did not materially change. Her chart follows:

CASE OF MRS. "R," AGE 23 YEARS

Date	Wt. Kilos	Diet Calor- ies**	BLOOD ANALYSIS		URINE ANALYSIS*					
			Sugar Per Cent	CO ₂ Combin- ing Power of Plasma	Vol. C. C.	Sp. Gr.	Sugar Grams	Acce- tone	Dia- cetic Acid	Indi- can
***9/19	53.2	R	0.360	68	2600	1037	78	++++	++++	++
9/20	51.4	R			3160	1047	126.4	++++	++++	+
9/21	53.0	R			2600	1040	104	++	++++	+
9/22	52.3	R			3000	1042	150	++	++	Neg.
9/23	53.2	R			3200	1040	160	++	++	+
9/24		R			3500	1042	175	++	++	+
***9/25	54.4	R	0.36	62	3650	1040	240.9	++	+	+
9/26	54.1	F. F.			2200	1040	110	Trace	Trace	Trace
9/27	54.0	A. T.			650	1020	Neg.	++	++	Neg.
9/28	53.4	A. T.	0.120		800	1022	Neg.	++	++	Neg.
9/29	53.6	54			950	1027	Neg.	++++	++++	Trace
9/30	53.0	234			1200	1024	Neg.	++++	++	+
10/1	52.3	354			720	1026	Neg.	++++	++++	++
10/2	53.2	504	0.120	52	700	1026	Neg.	++++	++++	++
10/3	54.1	631			850	1026	Neg.	++	++	Trace
10/4	55.0	823			800	1029	Neg.	++	++	++
10/5	55.7	1131			1800	1011	Neg.	V.F.T.	V.F.T.	Trace
10/6	54.4	1305			1400	1010	Neg.	V.F.T.	V.F.T.	Trace
10/7	54.2	1525			1300	1011	Neg.	Trace	Trace	Neg.
10/8	54.2	2023			950	1015	Neg.	V.F.T.	V.F.T.	++
10/9	54.4	1719	0.129		1100	1014	Neg.	V.F.T.	Neg.	Trace
10/10	54.2	1845			1400	1014	Neg.	V.F.T.	Neg.	++
10/11	53.9	1883			1250	1011	Neg.	Neg.	Neg.	++
10/12	54.3	1819	0.141		1100	1016	Neg.	Neg.	Neg.	++
†10/13	54.9	1859			1200	1015	Neg.	Neg.	Neg.	+
††10/30						1022	Neg.	Neg.	Neg.	Neg.

*++++=Large amount.

++=Moderate amount.

+=Small amount.

V.F.T.=Very faint trace.

**R=Regular mixed diet.

F.F.=Fat-free diet.

A.T.=Starvation.

***During above period patient was given 1-10 grain of parathyroid three times a day. Note increase in urine sugar.

† Patient left hospital.

††Urine received by mail.

This patient has been heard from several times. She is now taking over 2,000 calories and sugar has reappeared but once in her urine. Under one day's starvation, this quickly disappeared. Since then she has been sugar-free. No opportunity has been had since to obtain her blood for examination. This might be termed a very successful issue under the Allen treatment.

The next case, that of Mr. W, represents what might be termed an unsuccessful case. This man, aged 55 years, married, displayed nothing in his family history to point to diabetes, no

obesity, gout or tuberculosis in father, mother, brothers, sisters or other relatives. He was an occasional drinker, moderate at venery, formerly did a good deal of manual labor, sleeps well. Three and a half years ago began to lose weight and developed polyuria, gradually developing polyphagia and polydipsia. Sugar was first discovered in his urine three years ago on account of having consulted his physician because of his polyuria and loss in weight. So far as etiological factors are concerned, he had been addicted to dietary excesses. He gave a negative Wassermann and Hecht-Gradwohl test for syphilis, had never had any trauma, had occasional pains in the region of the pancreas but no palpable tumor. There was no disturbance in the thyroids, no symptoms of gout (blood uric acid was normal in quantity), and no hypertension. His weight on coming under observation was 101 lbs., height 5 feet 8 inches, marked loss of strength, marked polyuria, polyphagia, pains over pancreatic region, had numbness in legs, cramps in lower legs, had lost all teeth six months before (pyorrhea alveolaris), bowels constipated, had occasional headaches, coughed frequently, examination of lungs disclosed evidences of beginning tuberculosis of left lung, confirmed microscopically. A very much emaciated man, with pale visible mucosæ, thyroid normal, slight delay in contraction of pupils, hearing good, breath gave acetone odor, arteries soft. Diagnosis: Diabetes mellitus and pulmonary tuberculosis. His urine showed 97.3 grams sugar in twenty-four hour specimen of 2950 c.c. His blood showed 0.280% sugar. (See chart on page 163 for full facts of this study.)

He was under observation forty-three days. He was tried out on the Allen treatment but responded very poorly. The highest amount of calories he could take without producing glycosuria was 1060—clearly insufficient to maintain life. He was in a state of acidosis at the very beginning of his observation, showing a carbon dioxide combining power of but 50, with marked amounts of acetone and diacetic acid in his urine. Every attempt was made to prevent acidosis and to keep him sugar-free and at the same time give him sufficient nourishment to support life, but this was never successfully consummated. He finally left the hospital showing a persistent hyperglycemia, and a trace of sugar under 1060 calories of food. He was apparently doing very badly un-

CASE OF MR. "W," AGE 55 YEARS

Date	Wt. Kilos	Diet Calories**	BLOOD ANALYSIS		URINE ANALYSIS*					
			Sugar Per Cent	CO ₂ Combin- ing Power of Plasma	Vol. C. C.	Sp. Gr.	Sugar Grams	Acet- one	Dia- cetic Acid	Indi- can
10/1		R			3100	1036		+	+	Neg.
10/2	46.0	R	0.280	50	2950	1040	97.3	+	+	+
10/3	46.0	F. F.			1400	1038	70.0	++	++	Trace
10/4	46.0	A. T.			800	1018	+	++	++	Trace
10/5	45.5	A. T.			1400	1017	+	+	+	Trace
10/6	45.0	A. T.	0.200		800	1020	Trace	+	+	Trace
10/7	44.3	A. T.			950	1015	Trace	Trace	Trace	Neg.
10/8	44.6	I. S.			1200	1014	Trace	Trace	Trace	Trace
10/9	44.6	A. T.			520	1020	+	Trace	Trace	Trace
10/10	42.5	A. T.			740	1017	V.F.T.	Trace	Trace	Trace
10/11	43.9	A. T.			1600	1010	Neg.	Trace	Trace	Trace
10/12	42.4	35	0.200	49	800	1016	Neg.	Trace	Trace	Trace
10/13	42.4	220			900	1015	Neg.	Trace	Trace	Trace
10/14	42.2	360			600	1015	Neg.	Neg.	Neg.	Trace
10/15	43.4	462			1100	1016	Neg.	Neg.	Neg.	Trace
10/16	43.4	542			950	1015	Trace	Neg.	Neg.	Trace
10/17	43.9	734	0.200		1600	1015	Trace	Neg.	Neg.	Trace
10/18	43.4	A. T.			1100	1015	Neg.	Neg.	Neg.	Neg.
†10/19	42.8	195			520	1016	Neg.	Trace	Trace	Neg.
10/20	43.4	140			1000	1016	Neg.	Neg.	Neg.	Trace
10/21	43.8	370			650	1017	Neg.	Neg.	Neg.	Trace
10/22	43.4	478			300	1023	Neg.	Neg.	Neg.	Neg.
10/23	43.4	602			750	1020	Trace	Neg.	Neg.	Trace
10/24	43.2	A. T.	0.156	55	600	1018	Neg.	Neg.	Neg.	+
10/25	42.9	600			850	1020	Trace	Neg.	Neg.	Trace
10/26	43.6	629			1000	1020	Trace	Neg.	Neg.	Trace
10/27	43.1	A. T.			900	1020	Trace	Neg.	Neg.	Trace
10/28	42.8	A. T.			850	1018	Neg.	Neg.	Neg.	Trace
10/29	42.6	354			1100	1015	Neg.	Neg.	Neg.	+
10/30	42.8	472			900	1018	Neg.	Neg.	Neg.	Trace
10/31	43.5	609			950	1022	Neg.	Neg.	Neg.	Trace
11/1	42.9	866	0.189	54	800	1020	Neg.	Neg.	Neg.	Trace
11/2	43.6	1038			1200	1015	Neg.	Neg.	Neg.	Neg.
11/3	43.0	1044			870	1018	Trace	Trace	Trace	Trace
11/4	43.1	A. T.			1050	1015	Neg.	V.F.T.	V.F.T.	Trace
11/5		751			1200	1015	Trace	Trace	Trace	+
11/6	43.1	A. T.	0.192		700	1016	Neg.	V.F.T.	V.F.T.	Trace
11/7	42.6	A. T.			550	1015	Neg.	Neg.	Neg.	Trace
11/8	42.7	592			1200	1016	Neg.	Neg.	Neg.	Trace
11/9	43.0	939			750	1020	V.F.T.	Neg.	Neg.	Trace
††11/10	42.8	1058			1100	1020	Trace	Trace	Trace	Trace
††11/11	43.4	1060			1200	1022	Trace	Trace	Trace	Trace
11/12	Patient	Left	Hospital.							

*++++=Large amount.
 ++=Moderate amount.
 +=Small Amount.
 V.F.T.=Very faint trace.

**R=Regular mixed diet.
 F.F.=Fat-free diet.
 A.T.=Starvation.
 I.S.=Intermittent starvation.
 †Patient fed by mistake.
 ††Patient eating outside.

der the treatment; besides, his tuberculous infection seemed to be making fast inroads upon his general condition. This failure of the Allen treatment, of course, occurred in a case that was both an advanced diabetic and a rapidly advancing pulmonary tuberculous subject. The tuberculosis infection naturally had impoverished his system and prevented a fair trial of the treatment. We narrate the case, however, as a very good example of a study of blood and urine in complicated diabetes mellitus.

CHAPTER XXVI.

ACIDOSIS.

We will now consider acidosis, its cause, its symptomatology, its significance, its recognition by blood and urinary findings. In acidosis it is not meant that the reaction of the blood actually changes from its alkaline or neutral reaction to acid reaction. This is impossible, for life can not be sustained if an acid condition of the blood occurs. In the very last stages of life, practically *in extremis*, an acid condition of the blood occurs, but under no other circumstances.

It must be remembered that the neutrality of the blood depends upon the mixture of carbonic acid, carbonates, and phosphates in the blood and that these seem to remain at constant values even though the exogenous source of alkalies or acids is increased or diminished. This was shown by Henderson.¹ Carbon dioxide is also thrown off from the lungs and the urine in health is acid in reaction; this helps in maintaining the alkalinity of the blood. The physiology of the respiratory center is most interesting for when the amount of acid increases in the body, there is a quick stimulation of these centers with the result that more CO_2 is thrown out and the acid condition of the blood is prevented from assuming larger proportions. Any excess of acids induces this phenomenon. When the acidity of the blood is threatening, there is a quick call on the ammonia. It is only when the ammonia is being used up, that "acidosis" supervenes. In the course of normal metabolism we know that the ammonia of the body is converted into urea and eliminated as such, but the supervening acidosis takes up some of this ammonia and keeps the blood alkaline. Application of principles calling for an estimation of the alveolar carbon dioxide tension of course gives valuable information about acidosis. In a very recent publication, Marriott² has called attention to a simple method for the de-

¹Henderson: *Ergeb. d. Physiol.*, 1909, vol. viii, p. 254; *Science*, New York, 1913, vol. xxxvii, p. 389.

²Marriott: *Jour. Am. Med. Assn.*, May 20, 1916.

termination of this tension. We shall fully cover this later.

Howland and Marriott³ assert that the term is loosely used, that acidosis is spoken of when acetone bodies appear in the urine. This is not necessarily true. We must remember that the regulators of the alkalinity of the blood are (1) sodium bicarbonate, occurring in plasma and cells, (2) the acid and alkaline phosphates of sodium and potassium found in the red blood cells, and (3) the proteins. Acid in the shape of carbonic acid is formed in the tissues. Respiration lowers the concentration of CO_2 in the lungs and allows the higher concentrations in the tissues to escape into the lungs and be removed. Concentration is highest in the tissues, lower in the blood, and lowest in the lungs. Henderson⁴ calls carbonates of the blood the first line of defense against acidosis. Dyspnea or hyperpnea, or increased pulmonary ventilation, is the greatest aid for the liberation of carbon dioxide from the body.

A second line of defense is the capacity of the kidneys to excrete an acid urine from a neutral blood. They remove acid phosphate and save base with each molecule of acid phosphate that they excrete. A third line of defense is furnished by the proteins. Proteins can combine with appreciable amounts of either acids or alkalies without undergoing any marked changes in reaction. Another line of defense is the ammonia of the body. The body can neutralize acid by producing ammonia. This occurs at the expense of the urea. Aside from the interest we have in acidosis as part and parcel of our study of diabetes mellitus, acidosis occurs in children in connection with other conditions.

Quoting from Howland and Marriott:⁵ "Even when no evidence of disease can be detected to which the acidosis can be referred, acidosis may be found. For instance, a boy of six was suddenly taken ill with high fever. Inside of twelve hours he was brought to the hospital with great dyspnea of the air-hunger type. Physical examination was quite negative except for a purulent otitis media. All the tests made indicated acidosis. The bicarbonate of the blood was greatly reduced. The reaction

³Howland, John, and Marriott, W.: Bull. Johns Hopkins Hosp., March, 1916, vol. xxvii, No. 301.

⁴Henderson: Am. Jour. Physiol., 1908, vol. xxi, p. 427.

⁵Howland, John, and Marriott, W.: Bull. Johns Hopkins Hosp., March, 1916, vol. xxvii, No. 301.

of the blood had shifted markedly toward acidity and yet the acetone bodies in the blood were not greatly increased. The tolerance for alkalies was enormously increased. Though he took by mouth 20 grams of soda and 6 grams by rectum without vomiting or diarrhea, no change in the reaction of the urine was produced thereby. But the alkalies had a profound influence upon his condition; his respirations diminished in rapidity and depth, the evidences of acidosis to be obtained by the various tests rapidly disappeared and he made an uninterrupted and apparently complete recovery; for he now seems entirely well and has been so for six months.

“We may then say that acidosis is not an uncommon condition in infancy and childhood; that while it is especially frequent in the severe diarrheas of infancy, it may appear with a variety of diseases, and sometimes, apparently, alone. To recognize it with older children is not very difficult. The character of the respiration is usually sufficient to arrest one’s attention and one or two relatively simple laboratory tests will quickly determine the question one way or the other. With infants who are irritable, restless and crying, it is much more difficult to say whether hyperpnea is present; and yet with them it is most important to make the diagnosis early, for the reason that acidosis is such a fatal complication of diarrheal disease in infancy. Older children react promptly and often permanently to alkali therapy. It may be possible to stop the clinical and laboratory evidences of acidosis in infants, but the patients usually die. Why they do cannot be determined at the present time. Many normal processes have undoubtedly been inhibited, perhaps permanently, and many abnormal ones stimulated. A restoration to normal conditions seems nearly impossible. For this reason we should not wait until acidosis can be demonstrated. From the beginning we should give bicarbonate of soda to infants with severe diarrhea in sufficient quantity to render the urine alkaline and keep it so.

“We may lay it down as a general maxim that as hyperpnea indicates acidosis, so hyperpnea indicates alkali therapy, and this for infants or older children. The alkalies may be given by mouth, by rectum, subcutaneously, or intravenously. Vomiting and diarrhea frequently render their administration by mouth or by rectum out of the question. Then one of the other methods

must be employed. Intravenous administration is the method of choice, especially when rapidity of action is desired—and with acidosis rapidity of action is always desired.

“The superior longitudinal sinus, as advised by Marfan, Tobler and Helmholtz, is available with infants, or the external jugular or femoral veins. With older children, a vein in the arm can often be employed. If facilities for the intravenous injection of alkali are not at hand, the injection may be made subcutaneously, with care that the bicarbonate has not been transformed into the carbonate, else severe sloughing of the tissues may result. A four per cent solution is usually employed for intravenous use and a two per cent solution for subcutaneous use. The quantity to be injected depends upon the size of the child, the severity of the symptoms and the effect produced, but the amount is always large. It must be given until the urine becomes alkaline; even in infants under one year, as much as 10 gm. in 24 hours may be required.

“With the cases of acetone-body acidosis with no sugar in the urine and with a low sugar content in the blood, glucose by rectum, subcutaneously or intravenously, seems clearly indicated in addition to the alkali. With all forms water is urgently required, especially with infants who are dessicated as a result of the vomiting and diarrhea.

“Much remains to be learned regarding acidosis. The presence of abnormal acids explains the origin of some forms, but there are others that are in nowise understood. Are there abnormal acids whose presence has not been detected? Are normal acids formed in excess? Are bases lost? Does the kidney fail to excrete sufficient acid? These are a few of the questions at present unanswered that must be answered before our knowledge of acidosis can be considered in any way complete. Much has been learned in the last few years; with the present greatly stimulated interest in the subject, we may confidently expect that the future will provide answers to many of the questions that now seem obscure.”

Our interest in acidosis is intimately connected with the diabetic where the sugar can be utilized and the acetone bodies accumulate in the blood. The study of the hydrogen-ion concentration of blood will throw light on diabetic acidosis: Marriott has pointed out a method for this study (see page 66). The ear-

bon dioxide tension of alveolar air should also be studied; Marriott's method determines this and thus estimates the degree of severity of the acidosis and the results of the treatment of the same. This is a very excellent way of arriving at such a conclusion, but, it must be remembered as Marriott states in his monograph,⁶ that, "Changes in the pulmonary epithelium such as would prevent the air in the lungs from coming in equilibrium with the blood in the capillaries, would, of necessity, affect the composition of the alveolar air. Since very little is known as yet regarding the exact effect of such changes, one is hardly justified in drawing conclusions regarding acidosis from the composition of the alveolar air in patients with pulmonary affections."

The neutralization of the acidity that threatens in acidosis occurs also through the ammonia reserve, as alluded to above. It has been repeatedly stated by writers on the prevention of acidosis that the consumption of fats must be stopped, in fact, in the preliminary preparation of a patient for the Allen treatment, fats must be excluded so as to prevent or lessen the chance of acidosis from long-continued fasting. Why is this true? The metabolism of fats will easily explain this: in the absence of the proper carbohydrate balance or tolerance (which is the situation that exists in severe diabetes) the substances that result from the cleavage of the higher fatty acids (such as stearin, palmitin) of fat, are transformed into oxybutyric acid and diacetic acid, instead of pursuing the normal path of transformation into butyric acid. There is no further oxidation. Also these acids, oxybutyric and diacetic, may arise from certain of the amino-acids, leucine, tyrosine, phenylalanin, which occur when protein is split up. These organic acid derivatives of the fat and protein matter of the body furnish the basis for the formation of the so-called acetone bodies which are *acetone*, *beta-oxybutyric acid* and *diacetic acid*. Their formulas are as follows:

When these bodies appear in the blood in excess we have acidosis, but it must again be stated that they do not produce an acid reac-

⁶Marriott: Jour. Am. Med. Assn., 1916, vol. lxvi, p. 1594.

tion of the blood. When they are excreted in the urine we speak of ketonuria or acetonuria. As a matter of fact no acetone is eliminated as such by the kidneys: they do eliminate diacetic acid, but from this acetone is formed in the urine. This chemical formation is easy to follow; it simply consists in the diacetic acid throwing off the molecule COOH , resulting in acetone. Emphasis must be laid upon the fact that acidosis does not occur when the body is easily and normally burning up its sugar. It is when it can no longer do so, that the chemical processes already explained occur. The fats under normal condition are burned up in the elaboration of the carbohydrate metabolism, but when the carbohydrate metabolic processes are in abeyance, then the fats go through their imperfect evolution to diacetic acid and beta-oxybutyric acid and acetone, i. e., acidosis then occurs.

We have called attention to the fact that the ammonia is called upon to "suppress" the acidosis. One of the methods for determining the ammonia output which in turn will guide us in estimating the degree of acetonuria, is to determine the amount of bicarbonate of sodium necessary to render the urine alkaline or amphoteric. Normally from 5 to 10 grams of bicarbonate of sodium will render the urine alkaline. In mild acidosis, 20 grams are required; in severer cases from 30 to 40 grams; and in extreme cases 40 grams or more. In coma, when urine is excreted, it is usually impossible to neutralize the urine or make it amphoteric, no matter how much sodium bicarbonate is used.⁷

Another method, however, which is a much more delicate test for acidosis than any of the urine tests or the sodium bicarbonate test just described, is the estimation of the carbon dioxide combining power of blood plasma, as described by Van Slyke. Here we have a ready method for exactly and quickly determining the ability of the patient's blood plasma to take up carbon dioxide. When the ability of the patient's plasma is impaired in taking up carbon dioxide, then we have an acidosis. Thus blood plasma, normally, has the capacity to combine with 65 per cent or more of the carbon dioxide, which can be thrown into it in the form of alveolar air. When this percentage falls below 50, we must consider the individual in a state of acidosis. This method is equal in efficiency to the methods of determination of the blood

⁷Barker: Monographic Medicine, vol. iv, p. 820.

hydrogen-ion concentration of Marriott or the method of determination of the carbon dioxide tension of alveolar air. The characteristic readings on the Van Slyke apparatus are anywhere below 50 in marked acidosis. Thus the carbon dioxide combining power in a case of diabetes has been seen to drop from 50 to 30. The administration of alkalies has a profound influence upon it. This brings us to a short consideration of the use of physical and chemical forces in combating this condition. Inasmuch as the acetone bodies result from the imperfect and incomplete breaking up of the fat molecule, it is rational to interdict the use of fats. Secondly, the condition occurs as a result of imperfect carbohydrate metabolism. The glucose is not being burnt up. We try to burn up the carbohydrates. It is said that alcohol assists in the burning up of glucose, and therefore should be tried. Since alkaline substances taken into the body will help to render the urine amphoteric, we must quickly throw into such a case as much sodium bicarbonate as possible. As much as a teaspoonful every half hour in water should be given to a patient with impending diaceturia until his urine becomes amphoteric.

Marriott, Levy, and Rowntree⁸ have described their method for determination of the hydrogen-ion concentration of the blood, as given on page 66.

It might be advantageous to amplify their work here regarding the variations in the hydrogen-ion concentration of the blood. They maintain that human blood as it exists in the body is faintly alkaline in reaction, that is, it has a hydrogen-ion concentration only slightly less than that of pure water, and this degree of alkalinity tends to be maintained even when considerable quantities of acids are produced within the body, or are introduced from without. Acidosis may be recognized in various ways, by an increase in the ammonia coefficient in the urine, decrease of carbon dioxide tension of alveolar air, the finding of abnormal acids in the blood and urine, increased alkali tolerance and by diminished titratable alkalinity of the blood serum, by changes in the hemoglobin dissociation curve and by actual determination of the hydrogen-ion concentration of the blood. A change in the hydrogen-ion concentration of the blood indicates a failure of the

⁸Marriott, Levy, and Rowntree: *Arch. Int. Med.*, 1915, vol. xvi, p. 388.

protective mechanism and the onset of acidosis. It is in this connection that the determination of the hydrogen-ion concentration of the blood according to the technic given on page 66 is of value. With the use of this method, a series of bloods from normal and pathologic cases were studied with the following results:

1. Normal individuals: twenty-five cases. A. Serum; twenty-four of the twenty-five cases read between 7.6 and 7.8, in one instance 7.9 was the record:

pH	Cases
7.6	4
7.65	1
7.7	5
7.75	5
7.8	9
7.9	1

B. Whole blood (oxalated by collection in tubes containing a little dry powdered sodium oxalate, free from carbonate); nineteen determinations. These all read between 7.4 and 7.6:

pH	Cases
7.4	3
7.45	2
7.5	4
7.55	5
7.6	5

The slightly greater acidity of whole blood as compared with serum has been recognized by others and is due possibly to the fact that hemoglobin and especially oxyhemoglobin, behaves as a weak acid.

C. Defibrinated blood: These writers used early in their work defibrinated blood run in parallel series with serum and oxalated whole blood. No additional information was gained by using defibrinated blood, it complicated the work and so its use was abandoned.

2. Miscellaneous medical cases were studied, sixty-three determinations in 52 cases, comprising the following diseases: nephritis, acute and chronic, diabetes mellitus, myocardial insufficiency, syphilis, arthritis, tuberculosis, etc. With respect to the serum of these cases, sixty of the sixty-three determinations read between 7.6 and 7.8. With whole blood, thirty-three determinations gave thirty-one between 7.4 and 7.6.

3. Acidosis cases were studied, eight cases with fifteen determina-

tions. The general conclusions respecting the value of this method of estimating acidosis are as follows:

A. The indicator method of determining hydrogen-ion concentration is made applicable to blood and serum by utilization of dialysis through a collodion membrane, which excludes the disturbing influences of color and of proteins. The method is simple, accurate, rapid, and well adapted for clinical work.

Fig. 62.—Fridericia apparatus for determination of carbon dioxide in alveolar air.

B. The technic consists of dialyzing 3 c.c. of blood or serum at room temperature against 3 c.c. of 0.8 per cent salt solution for five minutes, adding an indicator and comparing with colored standard phosphate mixtures of known hydrogen-ion concentration.

C. Phenolsulphonphthalein is employed as the indicator in this

method. It is found to exhibit easily distinguishable variations in quality of color, with minute differences in hydrogen-ion concentration between the limits of pH6.4 and pH8.4.

D. Oxalated blood from normal individuals gives a dialysate with a pH varying between 7.4 and 7.6, while that of serum ranges from 7.6 to 7.8.

E. Variations from these figures toward the acid side were encountered only in conditions which clinically, and from the standpoint of laboratory findings, evidenced an acidosis.

F. In a small series of clinical acidoses, the serums varied from 7.55 to 7.2 and oxalated blood from 7.3 to 7.1. In experimental acidosis in dogs, a pH of 6.9 was encountered in both serum and blood just before death.

A method for determination of carbon dioxide in alveolar air is that of Fridericia (Fig. 62). This method does not involve the use of expensive apparatus, can be transported to the bedside, and only occupies about fifteen minutes. It requires the cooperation of the patient and consequently cannot be used when the patient is in coma, but when this occurs the Van Slyke and urinary findings will suffice. Fridericia⁹ described his method in 1914. Horner¹⁰ describes the method as follows:

“This method possesses the advantage of being simple and involving the use of apparatus which may be easily transported to the bedside. One hundred cubic centimeters of alveolar air are collected in a closed chamber and then cooled from the temperature of the body to that of the room. The carbon dioxide in this air is then absorbed with a 20 per cent aqueous solution of potassium hydrate, thereby creating a partial vacuum, which in turn is equalized with water. This water is then subjected to atmospheric pressure, when the amount of carbon dioxide replaced by water can be read in percentage of atmospheric air by reading the height in centimeters to which the column of water has risen in the closed 100 c.c. chamber. This percentage may be changed to millimeters of mercury pressure by multiplying the difference between barometric pressure at the time of the test, and this varies in Boston between 700 mm. and 750 mm., and the tension of aqueous vapor at 37.5° C. which is 48 mm. mercury.

⁹Fridericia: Berl. klin. Wchnschr., 1914, p. 1268.

¹⁰Horner: Boston Med. and Surg. Jour., 1916, vol. clxxv, No. 5.

This will make a factor which lies between 718 and 702. As the reading of 760 is much the more common at sea level, for clinical purposes the factor 715 may be used satisfactorily. The patient should be in the same position and quiet for ten minutes prior to the performance of the test.

“After a normal inspiration, the end (*A*) of the apparatus is inserted between the lips, and the patient is instructed to expire forcibly through the apparatus, with cocks *C* and *D* open, so that there is a free passage from *A* to *B*. The tube remains in the mouth throughout the entire expiration and the cock *C* is then closed, thus retaining between cocks *C* and *D* the last 100 c.c. of expired air. (As the exchange of air in the upper respiratory passage is 200 c.c. and the exchange of air from the alveoli is 800 c.c., it is plain that with any care at all a sample of alveolar and not upper respiratory air will be obtained.) The apparatus is now immersed in a glass tank of water at room temperature and allowed to remain there five minutes. The best way to obtain water at room temperature is simply to keep the glass tank in the room with the patient for several hours before the test, though with an ordinary thermometer one can easily adjust the temperature of the water to that of the room. At the end of five minutes, about 10 c.c. of 20 per cent aqueous solution of potassium hydrate is poured into the apparatus through the orifice *B*. A little of this potassium hydrate will leak through the hole in cock *D* to chamber *CD*. Now cock *D* is turned to the left so that chamber *CD* is closed and chamber *BD* is also closed. The small amount of potassium hydrate in chamber *CD* is shaken in the chamber for a moment. Then with apparatus in upright position, cock *D* is turned so that there is a continuous passage from *C* and *B*, and the amount of potassium hydrate which will run into the chamber *CD* is allowed to do so. Now cock *D* is turned to the left until *BDE* is a continuous passage, and in this way potassium hydrate is allowed to escape into the water tank. Chamber *CD* still contains 2 or 3 c.c. of potassium hydrate solution and should be thoroughly washed with this solution. Every point in the surface of chamber *CD* must be touched by the alkaline solution. This is accomplished by shaking very thoroughly the potassium hydrate in chamber *CD*. The apparatus is again

immersed in the tank of water, cock *D* is turned to the left until water rises into *CD* through *EDC*, and the apparatus left in the water five minutes. At the end of this time, the apparatus is raised until the bottom of the meniscus of the water in chamber *CD* is level with the top of the water in the tank. Now cock *D* is turned to the right until water runs through *EDB* to the level of water in chamber *CD*, which is now closed. Then cock *D* is turned further to the right until *CDB* is a continuous chamber. The apparatus is then again immersed to the bottom of the glass tank and the water in the arm *BD* of the apparatus should be at the same level with the water in the chamber *CD* and continuous with it. If this is not so, then the amount of the water in *BD* should be changed until it reaches the height of the column of water in *CD*. The reading is now taken in centimeters of the height to which the column of water stands in *CD*, and this is so graduated as to represent the percentage of CO_2 which was absorbed by alkali and replaced by water. This completes the test.

“The apparatus is prepared for the next test by opening cock *C* so that *A* to *B* is a continuous passage. The fluid in the apparatus is allowed to escape. Orifice *B* is put under the faucet and cold water allowed to run through the apparatus, taking care to shake sufficiently at the time so that water touches all of the inside of the apparatus. Repeat. Then pour through orifice *B* about 10 c.c. of 4 per cent solution boric acid. Rinse the apparatus very thoroughly with the acid so that there shall be no alkali remaining adherent to its sides. Wash again with cold water. Leave the apparatus so that orifices *A* and *B* are down, thereby allowing any water in the apparatus to drain out.”

From the above it will be seen that the necessary apparatus consists of the Fridericia appliance, a glass tank whose depth is equal to the length of the Fridericia apparatus, and a wash bottle containing 4 per cent solution of boric acid. It is convenient to add an indicator, such as alizarin, or litmus, to the alkaline and acid fluids.

Of the several methods recommended, the Van Slyke method of estimation of the carbon dioxide combining power of blood plasma is manifestly preferable, inasmuch as it does not entail the cooperation of the patient in its performance: an important

point when dealing with unconscious or semiconscious individuals. A comparison of the carbon dioxide tension in alveolar air by the Plesh method with the amount of carbon dioxide in the venous blood by Van Slyke's method has recently been published by Walker and Frothingham.¹¹ They collected the air for the method of Plesh,¹² as modified by Higgins,¹³ in the apparatus described in detail by Boothby and Peabody.¹⁴ In this method, as slightly modified by Boothby and Peabody, the patients could not always cooperate, yet they claim consistent results followed. In their use of the Van Slyke method they slightly modified the technic, i. e., instead of forcing alveolar air into the separatory funnel from the operator's lungs, they employed a separatory funnel of 250 c.c. capacity, which was filled from a spirometer with air of a known carbon dioxide percentage. Into this funnel 3 c.c. of the plasma was placed and shaken for two minutes. One c.c. of this mixture was then immediately put through the process already described on page 59. The figure obtained after being corrected for temperature and barometric pressure represented the number of milligrams of carbon dioxide in 1 c.c. of plasma. Van Slyke found that by multiplying this figure by the constant 35 he obtained a figure comparable to that obtained for the carbon dioxide tension in the alveolar air. Their observations were made on 100 different cases representing thirty different types of disease. A total of 116 observations in all were made. They found, for instance, that in primary anemia the carbon dioxide tension in the air varied in different cases by about 10 mm. The air and blood studied, however, did not vary more than three points. In a group of cases of Graves's disease, the carbon dioxide tension was slightly higher than the blood combining power, and in a few the difference was considerable. In typhoid fever the results were practically identical. In two cases of lung abscess the results were similar. In cases of chronic nephritis the results were practically alike. It was found that when the carbon dioxide tension was lowered in chronic nephritis, the combining power of the blood plasma was similarly lowered. In three cases of syphilis

¹¹Walker and Frothingham: Arch. Int. Med., Sept. 15, 1916, vol. xviii, No. 3, pp. 304-312.

¹²Plesh: Ztschr. f. exper. Path. u. Therap., 1909, vol. iii, p. 380.

¹³Higgins: Carnegie Inst. of Washington, 1915, p. 168, pub. 403.

¹⁴Boothby and Peabody: Arch. Int. Med., 1914, vol. xiii, p. 225.

the results were identical. Except in one case of cardiac disease with considerable emphysema, the studies were alike in cases of chronic cardiac disease. Even in cases of pneumonia where the respirations were hurried and the patients could not cooperate very well, the results were about the same. In acute articular rheumatism there were similar findings except that there was a difference in one case of as much as thirteen points. In five out of six cases of diabetes the air and the blood showed practically the same carbon dioxide tension. The sixth one showed a more marked variation, yet both determinations showed evidence of an acidosis, so that the variation in this case would not have been at all misleading. It is interesting to note that in all the cases of diabetes which showed acidosis, the blood was lower in carbon dioxide than the air. In other diseases the same story was told. In summing up the 116 observations, the carbon dioxide tension by the Plesh method corresponded with that estimated in the blood by the Van Slyke method. But little choice from the standpoint of accuracy can be offered with these two methods, but we recommend the Van Slyke method as being the simpler.

Summarizing, it may be stated that fasting for a normal individual is apt to be followed by acidosis quicker than for a diabetic subject. This is admirably seen in the Allen treatment, where fasting is *not* followed by acidosis, whereas in a normal individual in a few days he would begin to show the characteristic signs of blood and urine of acidosis and ketonuria. The body has certain safeguards against acidosis which are, the removal of acids from the blood through the lungs, the pulmonary action being increased by the stimulation of excessive acidity, and again the fact that there is a reaction between the molecule of disodium phosphate and a molecule of acid by which the sodium carbonate of the blood is conserved with the elimination of large quantities of acid. The amount of alkali in the body acts as a factor of safety against acidosis, in the form of sodium and potassium as well as the calcium and the magnesium of bones. We will call attention later on to this point in relation to the mineral metabolism of the urine. The factor of ammonia in the body must again be emphasized. This is due to the fact that the body can excrete nitrogen in the form of ammonia from the proteins, thereby converting some of the endogenous protein whose normal destiny is urea

into ammonia. It must be remembered that one gram of ammonia can neutralize five times as much beta-oxybutyric acid as one gram of sodium bicarbonate.¹⁵ The retention of the alkalinity of the blood is possibly best explained in Howland's own language.¹⁶ "The important constituents of the blood so far as the regulation of the reaction is concerned are (a) sodium bicarbonate, occurring both in the plasma and in the cells, (b) the acid and alkaline phosphates of potassium, found almost entirely within the red blood cells, and (c) the proteins.

"Considering the blood first as a solution of bicarbonates: a large amount of acid, carbonic acid, is constantly being formed in the tissues. It must be removed by the lungs, but first it must be transported to the lungs by the blood. This stream of acid which, with an adult, in the course of the day, is the chemical equivalent of several hundred cubic centimeters of concentrated hydrochloric acid, is sufficient to render acid any ordinary solution and keep it permanently acid. If this should happen in the blood, life would of course be impossible, but owing to the laws that govern the reaction of solutions of weak acids and their salts, the solutions of bicarbonate are able to take up a quantity of the acid, carbon dioxide, without appreciably undergoing a change in reaction. Thus there can be transported from the tissues to the lungs and so continuously eliminated from the body, a very large amount of acid. This steady escape of acid is accomplished with no harm and with no strain upon the organism. The respiratory center is adjusted to assist in the removal of the carbon dioxide. If there were no respirations and circulation were continued, eventually the carbon dioxide concentration would be the same in the tissues, in the blood and in the air and in the pulmonary alveoli.

"But the respirations lower the concentration in the lungs and thus allow the carbon dioxide to escape from the tissues where the concentration is highest by the blood where the concentration is lower, to the air in the lungs where the concentration is lowest. The respiratory center is extraordinarily sensitive to the slightest alteration in the reaction of the blood toward the acid side, so that an increased production of carbon dioxide in the

¹⁵Joslin: *Loc. cit.*, page 137.

¹⁶Howland: *Bull. Johns Hopkins Hosp.*, 1916, vol. xxvii, p. 63.

tissues, such as occurs, for instance, with muscular exercise, and the resultant slight excess in the blood is answered by an increased ventilation of the lungs which removes the carbon dioxide, thereby bringing the reaction of the blood back to normal. Other acids, whether formed in the body or introduced from outside, produce a similar effect. They displace the carbonic acid from the sodium bicarbonate and set the carbon dioxide free. This excess of carbon dioxide is removed by the increased pulmonary ventilation leaving a neutral salt, sodium oxybutyrate, or chloride, or what not, to be removed by the kidneys. Such a mechanism allows relatively huge amounts of abnormal acids to be at once rendered innocuous and removed; for instance, $\text{NaHCO}_3 + \text{HCl} = \text{NaCl} + \text{H}_2\text{O} + \text{CO}_2$. The hydrochloric acid is neutralized and the resultant sodium chloride is removed by the kidneys while the carbon dioxide is given off by the lungs.

"Henderson calls the carbonates of the blood the first line of defense. Thus, dyspnea, more properly hyperpnea or increased pulmonary ventilation, under abnormal circumstances, is an agent of the greatest value in ridding the body of carbon dioxide and thus keeping the reaction within normal limits. It may also be remarked that *hyperpnea* is the *best* of all the *evidences of acidosis* to be *obtained by physical examination alone*. It may almost be said that hyperpnea means acidosis.

"If the bicarbonates of the plasma were the only method of defense of the body, the organism would succumb to acidosis as soon as the bicarbonate was depleted by the excretion of neutral salts through the kidneys; every molecule of an acid would rob the body of a molecule of bicarbonate. The second mechanism here comes into play and is that by which acids may be removed leaving behind part of the base with which they have been combined, this base being available for further neutralization. The elimination is by way of the kidneys. These have the capacity to excrete an acid urine from a nearly neutral blood. They remove acid phosphate and save base with each molecule of acid phosphate that they excrete. Thus, although alkali is eliminated in the urine, it is much less than would be the case without this specialized kidney activity, and can readily be replaced under normal circumstances by the alkali of the food. For instance, with the introduction of a foreign acid— $\text{Na}_2\text{HPO}_4 + \text{HCl} =$

$\text{NaCl} + \text{NaH}_2\text{PO}_4$ —the hydrochloric acid is neutralized, the sodium chloride and acid sodium phosphate are excreted by the kidneys or the following reaction may take place— $\text{Na}_2\text{HPO}_4 + \text{H}_2\text{O} + \text{CO}_2 = \text{NaH}_2\text{PO}_4 + \text{NaHCO}_3$. By this method the sodium bicarbonate reserve of the body is renewed.

“Henderson and Palmer showed the magnitude of alkali sparing very prettily by titrating with alkali the acid urine back to the normal reaction of the blood. The alkali spared was found in normal subjects to vary in terms of tenth normal alkali, between 200 and 800 c.c. This is equivalent to saying that the kidneys eliminate from 200 to 800 c.c. of tenth normal acid in 24 hours.”

A very authoritative discussion on the question of fat in diabetes, in relation to acidosis particularly, is that of F. M. Allen, in his lecture before the Harvey Society of New York,¹⁷ entitled “The Role of Fat in Diabetes.” He showed the development of the methods of these problems by means of the new blood chemical tests which we have already described in Part I of this work. He said truly that it was a fine tribute to American science that every one of these tests was devised or perfected by an American investigator. Finally, the possibility of better study of the problems of diabetes was greatly increased by the ability to reproduce in dogs conditions almost identical with those encountered in human diabetes. This could be done by the surgical removal of a large proportion of the pancreas, leaving the remainder in communication with the intestine through the pancreatic duct. This operation rendered the dogs diabetic and yet retained their digestive functions through the preservation of the pancreatic secretion.

The first point in the problem of the role of fat in diabetes was that of lipemia. This condition was almost a constant finding in severe human diabetes and might be present to a slight degree even in very mild cases. The same was found to be true in partially depancreatized dogs. Further, diabetes in man and the partial depancreatization of dogs were the only conditions in which a high degree of lipemia was found. The fat might be present in the plasma of severe cases in either man or dog in amounts up to 15 per cent or over, and the ability to produce the

¹⁷Allen, F. M.: New York Med. Jour., Nov. 18, 1916.

condition in the latter afforded ideal conditions for the study of its causation and significance. It had long been believed that lipemia was due to a diminution in the lipase present in the blood, but this could now be stated to be incorrect and we could safely regard the lipase as quite a negligible factor. It had been shown in experimental dogs that lipemia varied in degree largely with the digestive power of the animals, that the fat was derived in great measure from the food fats, and that lipemia could be controlled largely by feeding. The fat in the blood was chiefly neutral fat with a considerable proportion of cholesterol, which ran parallel to the former, and a small amount of lecithin.

As to the causation of lipemia, experiments on the partially depancreatized dogs made it possible to say definitely: 1. Lipemia was not due to the occurrence of hyperglycemia. 2. It was not due to the absence of carbohydrate or to the loss of sugar. 3. It was not due to the presence of acetone bodies or to the change in the reaction of the blood. 4. It could not be produced by simple overfeeding with fat. Its exact cause is as yet unknown, but recent studies in the author's laboratory seem to point to its being related in some way to the condition of the cells in the pancreas, and evidence is accumulating which indicates that there may be an internal secretion of that gland which is directly concerned with the production of lipemia.

The second problem in the role of fat in diabetes is concerned with acidosis. Before entering into its discussion, it is necessary to have a clear understanding of what was meant by acidosis. In the author's opinion the term should be restricted to the original definition given by Naunyn, which stated that its one constant characteristic was the occurrence in the blood of an abnormal amount of beta-oxybutyric acid and acetone bodies. Contrary to the present misuse of the term it had nothing to do with a simple displacement of the reaction of the blood, and conditions with diminished alkalinity, increased carbon dioxide tension, increased hydrogen-ion concentration, and reduction of the "buffer" salts should not be classed as acidosis, since such a classification led to confusion.

Here, as in lipemia, the precise ultimate cause of acidosis is not known. It was fairly certain, however, that fat played an im-

portant role in its production and that the acids were produced largely in the muscles and liver—organs in which fat was burned. It was not yet known what proportion of fat could be burned without the production of acidosis in subjects with diabetes, or what proportion of carbohydrate was required to prevent the development of acidosis. It could be stated positively, however, that acidosis was not necessarily due to a lack of carbohydrate. If it was not possible to state the ultimate causes of acidosis at least the study of the partially depancreatized dogs had made it possible to gain an insight into some of the more remote causes.

It was found that acidosis could be produced in such dogs in three ways, all in complete imitation of the conditions encountered in man. First, it could be produced by following the plan adopted in the usual clinical treatment of human diabetes, namely, by giving a diet of high caloric value and high fat content. If an experimental dog with diabetes be made to hold or to gain weight—which is the practice in man—fat must be introduced into the dietary and calories must be crowded. One of two things soon happens in the dog; either he begins to vomit and suffer from diarrhea with loss of weight and refusal of the food, or, if the feeding is forcibly continued, his metabolism breaks down. When the latter occurs true acidosis develops and a fatal diabetic coma quite similar to that in man ensues. Such a diabetic coma can be produced in these animals while they are thus kept on a full diet, and this is just what occurs in human beings. Secondly, if the treatment employed in moderate human cases be applied to these dogs, the same results will ensue as in the first case. This is the fattening treatment which is marked by a reduction in the intake of protein and the administration of fat. These dogs look extremely well, but they go on to a fatal acidosis. The third way is that in which the animals are kept free from glycosuria through the administration of a diet very low in carbohydrates and consisting mainly of fat and protein. This form of diet is also often prescribed for man.

In both man and in these animals if the condition has not gone too far the acidosis may be checked by the introduction of a period of fasting, but if the diet is restored, the downward progress will continue. In severe cases—human or animal—the

fasting may at first increase the acidosis, but if the fasting is repeated with periods of return to a properly adjusted diet, it is usually possible to produce an immunity to the fasting acidosis and an ultimate recovery of very marked degree. These observations, along with others, the details of which cannot be given, all point to the existence of some specific internal function of the pancreas which is concerned with the production of acidosis. They also show that an alteration in the reaction of the blood is not the cause of death in acidosis, for the blood may be kept normal in reaction by the proper administration of alkalies, and yet the man or the animal may die of diabetic coma and typical acidosis.

If periods of fasting are properly introduced and the diet is adjusted, it is possible to keep the human or animal patient in a condition of physical comfort and fair health for long periods of time, and ultimately to increase his tolerance for foods to a great extent. It was also pointed out that the craving for carbohydrate seen in many diabetics was not due to "original sin," but was a physiological demand for that food element which does the most perhaps to control the development of acidosis. The same craving was to be observed in an intense degree in the dogs suffering from acidosis.

The last point to be discussed by Allen was the value of fat in the dietary of diabetics, and it was shown that fat unbalanced by other food constituents was a poison. The essence of these observations was to show that it was necessary to preserve a natural balance between fats on the one hand and protein and carbohydrate on the other if dangerous complications were to be avoided—especially acidosis and coma.

The net results of the observations pointed to the absolute necessity for clearing up the lipemia of diabetes; to the need of a proper appreciation of the importance of fat, unbalanced by other foods, in the production of acidosis; and finally to the most important fact of all, namely, that in diabetes there was a deficient assimilative function and that efforts to maintain the body weight by high calory feeding would soon lead to an exhaustion of whatever function remained to the patient. The true lesson to be learned was that it was not fat alone, not protein alone, and

not carbohydrate alone which was the source of danger, but that it was a disturbed balance between all three combined with an overtaking of the patient's assimilative powers which led to the downward progress of diabetics under the usual plans of dietetic regulation. Depending upon the severity of the case, the load on his assimilative function should be lightened; if he had acidosis he should be starved, once or repeatedly, until his assimilative function could be restored; and his diet should be kept within his assimilative capacity. If such a plan were followed, the majority of patients would live in comfort, and a large proportion would ultimately show a decided increase in the extent of their assimilative capacities.

In connection with the blood chemical methods for estimating acidosis in nephritis, the recent work of Marriott and Howland¹⁸ deserves special mention. They note that in the terminal stages of nephritis there is frequently an existing acidosis as determined by diminished carbon dioxide tension of the alveolar air, and increased hydrogen-ion concentration of the blood or serum, a diminution of the alkali reserve and of the oxygen combining power of the hemoglobin. They state that this acidosis is not due to an accumulation of the acetone bodies as they do not appear in the urine and they are not increased in the blood. That it is not due to the presence of lactic acid seems to be proved by the work of Lewis, Ryffel and others,¹⁹ who showed that lactic acid is not increased in the blood in this kind of acidosis. Henderson and Palmer²⁰ showed a diminished ammonia excretion in severe nephritis. An explanation for this acidosis of severe nephritis is the fact that the kidneys may be failing to excrete the acid substances which are ordinarily formed there. The regulation of the acid base equilibrium of the body is largely brought about by the ability of the kidney to excrete acid phosphate. In order to demonstrate whether or not this is true and whether or not in severe nephritis there is a consequent accumulation of inorganic phosphates in the blood, Marriott, Haessler, and Howland²¹ worked out a sim-

¹⁸Marriott and Howland: *Arch. Int. Med.*, Nov. 15, 1916, vol. xviii, No. 5, p. 708.

¹⁹Lewis, Ryffel, and others: *Heart*, 1913, vol. v, p. 45.

²⁰Henderson and Palmer: *Jour. Biol. Chem.*, 1915, vol. xxi, p. 37; *Arch. Int. Med.*, 1915, vol. xvi, p. 109.

²¹Howland, Haessler, and Marriott: *The Use of a New Reagent for Microcolorimetric Analysis as Applied to the Determination of Calcium and of Inorganic Phosphates in the Blood Serum*, *Jour. Biol. Chem.*, March, 1916, *proc.* xviii, vol. xxiv, No. 3.

ple method to determine these inorganic phosphates in a small quantity of serum.

This method is based upon the fact that the red color of a solution of ferric thiocyanate is discharged by certain substances, among which are oxalates and phosphates. Calcium is precipitated as the oxalate, dissolved in acid, added to a standard solution of ferric thiocyanate and made up to a definite volume. The color of the resulting solution is compared with that of a solution containing known amounts of calcium oxalate and ferric thiocyanate. The phosphates are precipitated as a magnesium and ammonium phosphate. The precipitate is dissolved and color comparisons are made as above.

In a personal communication, Marriott and Haessler give more elaborate details on this micro-determination of inorganic phosphates in the serum, as follows:

"Dilute 1 c.c. of clear, nonhemolyzed serum with 5 or 10 c.c. of water. Add two drops of N/10 HCl and 1 c.c. of 'magnesium mixture.'* Run in, drop by drop, with stirring, 2 c.c. of 10% ammonia (1 volume concentrated ammonia to 9 of water)—allow to stand over night at room temperature in order to complete precipitation. Filter off precipitate on a 10 c.c. Gooch crucible, the mat being prepared as follows: A small disc of filter paper is first placed in the bottom, asbestos soup is poured on to make a fairly thick mat,—another disc of filter paper is laid on and then a little more asbestos, finally a suspension of purified barium sulphate is poured on. This latter serves to make evident any leaks in the crucible and also to close the pores.

"Wash the precipitate four times, each time with 5 c.c. of the 10% ammonia,—then twice with 10 c.c. portions of 95% alcohol and finally twice with 10 c.c. portions of ether. The crucible is put back in the beaker and allowed to dry over night at room temperature or for an hour in an air bath at 50°. The washing with alcohol and ether is to remove lipoids.

"Ten c.c. of N/100 HCl is run into the crucible and the beaker

*Magnesium mixture is prepared as follows:

Magnesium chloride sticks,	10 gm.
Ammonium chloride,	5 gm.

Dissolve in 250 c.c. of water and add am-	
monium hydrate (conc.),	10 c.c.

Allow to stand over night to allow impurities to settle. Filter, neutralize with hydrochloric acid, and make up to 500 c.c.

covered tightly with a piece of rubber tissue secured with a rubber band and allowed to stand several hours to complete the solution of the precipitate. The asbestos is then thoroughly stirred up in the acid and the suspension poured off into a small tube and centrifuged. An aliquot portion (usually 6 c.c.) of the clear supernatant liquid is pipetted off and used for the determination.

“COLORIMETRIC COMPARISON.—*Ammonium Thiocyanate Solution* (3 grams to 1000 c.c. ferric chloride solution).—Weigh out 3 grams of ferric chloride with its contained water of crystallization. Dissolve in water and add just sufficient HCl to make a clear solution and make up to 1000 c.c. Just before use, these solutions are mixed by taking 5 c.c. each and making up to from 35 to 50 c.c. with distilled water, this solution being used more dilute for serum containing small amounts of phosphate. Accurately measured 2 c.c. portions of the iron thiocyanate solution thus prepared are measured into 10 c.c. volumetric flasks; the aliquot portions of the phosphate solution are added in the flask and the liquid made up to the mark with N/100 HCl. Known amounts of a standard solution of magnesium ammonium phosphate in N/100 HCl are added to other 10 c.c. flasks containing thiocyanate and made up to the mark with N/100 HCl. Color comparisons are made in small glass tubes approximately 120 mm. long by 10 mm., internal diameter. The tubes are filled to the same height and compared by looking through them lengthwise against a white surface. The colors do not change within an hour's time.

“*Standard Magnesium Ammonium Phosphate Solution*.—Dissolve .1584 gm. of *air dried* $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ in 100 c.c. of N/10 hydrochloric acid and dilute to 1 liter with water. Of this solution, 1 c.c. .02 mgm. phosphorus.

“Additional notes and cautions on the determinations of calcium and inorganic phosphate are given as follows:

“CALCIUM METHOD.—In the ashing of the blood by nitric acid a certain amount of difficultly soluble calcium sulphate is formed. This is especially insoluble if the liquid is allowed to go to dryness. In all cases, it is advisable after the nitric acid has evaporated to add distilled water to the flask and to heat on a sand

bath just below boiling for one hour or more, in order to completely bring the calcium into solution.

“By ‘20%’ sodium acetate is meant 20% of anhydrous sodium acetate. If the crystalline salt is used the solution should be 35%.

“The beakers used in the method should be of the tall, narrow type rather than of the broad form as in this way the solution of the precipitate seems to be more complete. Instead of centrifuging the asbestos suspension before removing an aliquot portion, filtration may be resorted to. Results obtained are the same.

“In the colorimetric comparison of calcium and of phosphates, instead of using 10 c.c. volumetric flasks, it is convenient to have a set of small flat-bottomed Nessler tubes, approximately 120 mm. long, 10 mm. internal diameter, these tubes being of exactly the same size and with a graduation at the 10 c.c. mark. The solutions may be made up in these tubes and mixed by inverting. In that way the volumetric flasks may be dispensed with.

“PHOSPHATE METHOD.—In making up the standard solutions, it is to be borne in mind that $\text{MgNH}_4\text{Po}_4 \cdot 6\text{H}_2\text{O}$ loses water of crystallization if heated, and, therefore, must be dried at room temperature. Commercial preparations of this salt are unreliable. It should be prepared by precipitation and dried as directed.”

By this method they determined the inorganic phosphates in the serum of a series of normal adults and older children and then of patients with nephritis, both with and without acidosis. The normal figure expressed in terms of phosphorus varied from 1 to 3.5 mgms. per 100 c.c. of blood. In the great majority of normals the amount was less than 2 mgms. They also determined the inorganic phosphate in the serum of patients with acidosis occurring in the course of nephritis and in every instance they found an increase in the phosphorus to many times the normal, that is, an increase up to 23 mgms. per 100 c.c. of blood. They believe that the retention of the acid phosphate (for approximately 90 per cent of the phosphate in an average urine is acid phosphate) would seem to be sufficient to account for the degree of acidosis observed. They do not claim that this is the sole factor in this acidosis of nephritis, but they point to the fact that the retention of acid phosphate in nephritis is not part of a general

salt retention; that it seems to be due to a certain "specificity" of retention because there was no corresponding increase of sodium chloride with the increase in acid phosphate. It was not proportional to the total nitrogen and the urea retention in these cases. In other words the phosphate retention was not a result of the acidosis *per se*, for these writers failed to find a similar increase in the inorganic phosphate in that form of acidosis seen in diabetics. They believe that the phosphate is due to some disturbance in the specific function of the kidney and not to increased phosphate production in the body or increased absorption from the intestinal canal, because the urinary output of phosphate is not increased and may even be decreased. They failed to reduce this phosphate retention by the administration of alkali and even demonstrated an increase of the substance under sodium bicarbonate administration.

They also found in these cases a marked reduction in the calcium of the serum, in one case as low as 1.5 mgms. per 100 c.c. of serum as compared with the normal of 10 to 11 mgms. The low calcium is to be referred to an excess of phosphates in the serum, as already detailed. The administration of phosphates causes an increased elimination of calcium through the feces, and the converse is also true; the administration of calcium leads to an increased elimination of phosphate, also by the intestines. This fact, according to these investigators, may offer a suggestion for rational therapeutic procedure.

At the May, 1916, meeting of the Association of American Physicians, a very excellent summing up of the entire question of acidosis was gone into by the leaders on this question; namely, L. J. Henderson, of Boston, John Howland, of Baltimore, R. T. Woodyatt, of Chicago, C. Frothingham, of Boston, L. G. Rowntree, of Minneapolis, Yandell Henderson, of New Haven, and Donald Van Slyke, of New York. It recapitulates most of what we have discussed, so we will abstract it here. In this symposium on acidosis,²² L. J. Henderson, speaking on the subject of the biochemistry of acidosis, said that like heat equilibrium, the equilibrium between acids and bases was essential to life. Fluctuations in equilibrium occurred, but normally the limits of fluctuation were narrow.

²²New York Med. Jour., Dec. 2, 1916, p. 1119.

Wider fluctuations occurred pathologically, but the acid base fluctuations did not as a rule involve changes in the hydrogen-ion concentration. Acidosis was defined as any disturbance of the acid-basic equilibrium whereby the power to resist acids in the body was lost. It is now possible to say that the main change in acidosis is the loss of blood bicarbonates. The bicarbonates were to be regarded as the *third* constituent of the blood; reckoning water first, salt second, and bicarbonate third. This third constituent is specially subject to fluctuations, owing to the constant physicochemical interchanges between blood and respired air; and since hydrogen-ion concentration is proportional to the reactions between bicarbonates and free carbon dioxide, the ratio of free carbon dioxide and bicarbonates is kept fairly constant by the mechanism of ventilation; hence, hydrogen-ion concentration is now regarded as the hormone of respiration. The maintenance of the acid basic equilibrium becomes more complicated in pathological states, and is always related to, and dependent on the general metabolism of the body. Beneath all metabolism is a constant diminution of blood carbonates; unless repaired, this leads to acidosis. The carbon dioxide tension of alveolar air and of the blood, together with the measure of alkali ingestion are the measures of acidosis. *Neither ammonia concentration nor urinary findings are safe guides.* Attempts to explain general pathological states on the basis of hydrogen-ion concentration or acidosis are not justified. Any attempt to treat a disease like nephritis by the indiscriminate administration of large amounts of alkali is malpractice. Small amounts of alkali, given over a long time, are allowable, and when so given, acidosis is impossible.

Howland, speaking on "Acidosis in Infants and Children," repeated some of the facts already credited to him in the preceding pages. He noted that acidosis in children is a dangerous, but not often, an acute, self-limited disease. It is not merely an acetonuria, but is dependent upon a loss of the acid basic equilibrium of the blood. Hyperpnea, as noted before, is the clinical sign; laboratory tests are the indices, these being carbon dioxide tension of alveolar air, hydrogen-ion concentration of blood, and alkali reserve of blood. The natural low level of carbon dioxide tension and low hydrogen-ion concentration in the young ex-

plains the susceptibility to acidosis. Onset of acidosis is marked by hyperpnea; coma soon ensues; the alkali reserve might be restored, but unless this occurs quickly, death follows. When acid phosphates are found in excess (five to fifteen times the normal) in the blood, and this condition continued long enough, it robs the body of its bases. Restoration of bases does not always stop the accumulation of acid phosphates. Acidosis is seen in many diseases of infancy and childhood and should always be kept in mind; its early, rational treatment may be the means of saving life.

The next paper in the symposium was that of R. T. Woodyatt on "Acidosis in Diabetes." He explained that the occurrence of acidosis in diabetes depended on the definition of the difference between the diabetic and the normal individual. The proportionality between glucose utilization and wastage depended upon the rate of intake. It may be said that with a rate of glucose intake high enough, the normal subject became diabetic; with the intake low enough, the diabetic acts like the normal individual. The difference was in the wastage. The occurrence of acidosis in diabetes depends upon this; for it has been found that one molecule of carbohydrate must be burnt to care for three molecules of higher fatty acids; if this ratio can be maintained, the body "smoked" with unburnt fats, acetone, beta-oxybutyric acid and diacetic acid appear in the urine. In diabetics the absolute rate of carbohydrate utilization is low and it is necessary to bend down the rates of protein and fat metabolism to meet that of the carbohydrates. Thus the application of rest, warmth and fasting in diabetes is rational. Acidosis in diabetes may be accounted for always in the way described, except in certain cases; e.g., its occurrence in the course of septic processes; such may be called accidental rather than diabetic acidoses.

Referring to "Acidosis in Acute and Chronic Diseases," Frothingham said that the finding of acidosis in diseased states other than diabetes led to a study of carbon dioxide tension of alveolar air, hydrogen-ion concentration in blood, acetone and ammonia nitrogen output in urine, and soda utilization in a large and diversified series of cases.

The very key-note of the discussion on acidosis was furnished

by Dr. Yandell Henderson, who emphasized the fact that in a discussion on acidosis, one writer speaks about one thing and another about an entirely different aspect of the question. There is need here, as in other medical discussions, of a clear cut nomenclature. It goes without saying that the acidosis of former days is not the acidosis of today. The acidosis of nephritis is not the acidosis of diabetes. Henderson urged that it might be better to speak in one case of a ketonuria and in another of low carbon dioxide states, and so on. In 1911 he was a member of Haldane's Pike's Peak expedition, and all of the party had acidosis when a sufficient altitude was reached, if the carbon dioxide tension was taken as an index. Henderson was very skeptical of the hurtful effects of acidosis, for he had seen no figures which indicated a more severe acidosis than he persistently had himself on Pike's Peak when feeling particularly well. The description given by Dr. Lawrence Henderson was on the basis of sea level data. But on going above sea level acidosis increased with the altitude; in a caisson, acidosis diminished. Miss Fitzgerald, of the Haldane expedition, had shown this as a result of hundreds of observations made by her at various altitudes. The net result of her work was that one could determine the altitude of any community by the measure of the carbon dioxide tension of the alveolar air of the inhabitants, or in other words, by their acidosis. It seemed, therefore, to Henderson, much safer to keep in mind the facts; from the urinary standpoint, acetonuria may be found; from the respiratory standpoint, variations in carbon dioxide tension, or volume of ventilation might be measured; from the point of view of the blood, disturbances of hydrogen-ion concentration might be noted; and other measures of the body's alkali acid balance might be made. But if all these measures were to be accepted as measures of acidosis, conditions of acidosis would be met with in which the acidosis was not a condition of acid blood at all, because the hydrogen-ion concentration of the blood might still be normal. It is therefore necessary to formulate and keep clearly in mind just what in the future is to be known as acidosis.

Van Slyke, concluding this very interesting discussion on acidosis, called attention to the fact that he and his co-workers had been much interested in the relations between the kidney, lung, and blood functions in acidosis and their observations had led them

to conclude that the phenomena arising in the various systems were the corollaries one to another. He had been struck by the beautiful concord between the clinical and the chemical facts, and the theoretical considerations advanced originally by Lawrence Henderson. Van Slyke thought that acidosis was a loss of the normal relationship between acids and the bicarbonates of the blood. He also believed in Rowntree's classification of compensated acidosis and true acidosis on the basis of undisturbed hydrogen-ion concentration respectively. He believed that the reduction of carbon dioxide tension of alveolar air is only an indirect measure of hydrogen-ion concentration of the blood and cannot be regarded as synonymous with acidosis. It is an exact measure of the hydrogen-ion state of the blood only when the lungs are functioning normally and under fixed conditions of temperature and atmosphere. The same may be said of the urinary findings: certain urinary changes are recognizable and acceptable as indirect evidences of acidosis: but they are not synonymous with acidosis, and depend upon renal integrity and other factors for constancy.

CHAPTER XXVII.

BLOOD CHANGES IN GOUT.

Among other conditions in which blood chemistry has played a role in differential diagnosis, might be mentioned gout and rheumatism. This disease which was most accurately described by Sydenham (London, 1763) is a peculiar condition about the etiology of which there still prevails much confusion. However, it may perhaps conservatively be stated at this time that it is a chronic disorder of metabolism in which there is an undue accumulation of uric acid in the blood as a result of a disturbance in the endogenous and the exogenous uric acid formation. Garrod¹ as long ago as 1848 contended that in gout we have an excess of uric acid in the blood due to increased formation and decreased elimination. Present-day methods of blood chemical analyses seem to prove that he was correct in his views, i. e., that in gout we have an undue accumulation of uric acid over the normal figure (1-3.0 mgms. per 100 c.c. of blood), whereas in rheumatism there is no such accumulation, the figure remaining around 1 to 3.0 mgms. Without going too deeply into the theories on the cause of this disturbance of metabolism, we might simply state that according to Brugsch and Schittenheim,² gout results from metabolic disturbances due to changes in the conversion of the purin bases. Folin and Denis³ showed that the amount of uric acid in the blood under normal conditions, using their colorimetric methods, varied from 0.7 to 3.7 mgms. per 100 c.c. of blood. Adler and Ragle⁴ reported, in 156 patients, a variation in uric acid from 0.7 to 4.5 mgms. per 100 grams of blood. These cases were taken at random from hospital cases and included conditions such as chronic interstitial nephritis in which there might be expected some increase in the normal amount of uric

¹Garrod, A. B.: *Med. Clin.*, 1848, vol. xxxi, p. 83; and *Treatise on Gout and Rheumatic Gout*, 1876.

²Brugsch: *Gicht. Spec. Path. u. Ther.*, 1913, Lieferung, I-IV, Wien u. Berlin.
Brugsch and Schittenheim: *Gicht*. Jena, 1910.

³Folin and Denis: *Jour. Biol. Chem.*, 1913, vol. xiv, p. 82.

⁴Adler and Ragle: *Boston Med. and Surg. Jour.*, 1914, vol. clxxi, p. 769.

acid. It was formerly supposed that uric acid could not be found in the blood of normal persons who were placed upon a purin-free diet. Its constant appearance with the patient on this diet was regarded in the nature of things as a test meal method of proving the existence of gout. That this was entirely erroneous has been proved time and again. For instance, McLester,⁵ using the method of Folin, found uric acid in the blood of fifteen normal individuals who had been on a purin-free diet for at least three days, in amounts varying from 0.5 to 2.9 mgms. per 100 c.c. of blood. Pratt⁶ showed the remarkable changes of uric acid in gout. He reported in 1913 eleven cases of typical gout in which the uric acid in the blood had been determined by the method of Folin and Denis in Folin's laboratory. In a subsequent paper he reports⁷ the number of cases studied as sixteen. He includes only cases in which tophi were found, or in which a history of characteristic attacks of acute gout was obtained or in which typical symptoms developed while under observation. Pratt's findings are quite interesting and deserve special mention. The average amount of uric acid irrespective of the diet or the condition of the patient at the time of the examination was 3.7 mgms. Three of the patients seen during the attacks were on an ordinary mixed diet. They had 4.5 mgms., 4.8 mgms., and 5.7 mgms. of uric acid. In the blood of two other patients examined during an attack while on a purin-free diet, the uric acid in four determinations ranged from 2.4 to 5.1 mgms., with an average amount of 3.6 mgms. None of these patients were taking atophan. Seven patients were examined at a time when they were free from symptoms of gout and when they were on a mixed diet. Their blood contained from 3.1 to 5.5 mgms. The average was 4.3 mgms. In the blood of six patients examined when they were on a purin-free diet and having no acute symptoms, Pratt found uric acid values from 1.6 to 7.2 mgms., an average of 3 mgms. These figures showed that in the cases studied there was more uric acid in the blood when on a mixed diet both in the interval and during attacks than when on a purin-free diet. In all, twelve examinations were made when a mixed diet was taken during attacks as well as in

⁵McLester: Arch. Int. Med., 1913, vol. xii, p. 737.

⁶Pratt: Tr. Am. Assn. Physicians, 1913, vol. xxviii, p. 387.

⁷Pratt: Am. Jour. Med. Sc., 1916, vol. cli, No. 1, p. 92.

the intervals, and the average amount of uric acid was 4.3 mgms. The general conclusion from these figures is that in gout there is always a hyperuricemia. Thirty-eight examinations made on sixteen cases of gout showed an average amount of uric acid of 3.7 mgms. per 100 c.c. of blood. It is generally believed that there is more uric acid in the blood during an acute attack than in the intervals, but this is not always true. Pratt's figures show, and other investigators corroborate them, that while in gout there is a relatively large amount of uric acid, the diagnosis of gout cannot be based absolutely upon a single blood test: there is a high concentration found at times in other joint conditions. But it must be remembered that in gout the condition of hyperuricemia is long-continued, while in the other joint conditions it is transitory. The obvious procedure, therefore, is to follow one examination up with others at interrupted intervals of time. For instance, one of Pratt's cases of infectious arthritis without any of the clinical features of gout, showed at the time of the first examination 7.6 mgms. of uric acid. Seven months later the blood was again analyzed and only 0.8 mgms. found, although the patient was then on a diet rich in purins. Other cases have shown the necessity of repeated blood examinations.

It seems that there is no relationship between the amount of uric acid retained in gout and the severity of the disease. Again, the age of the patient has no bearing upon this question. Attention must also be called to the fact that the retention of uric acid is in no way to be determined by a diminution in the output of uric acid in the urine. Vogt,⁸ Reach,⁹ and others have attempted to show that in gout the excretion of exogenous purin is diminished. Magnus-Levy,¹⁰ however, has disproved this completely, and Pratt's¹¹ figures show that a marked increase and retention of uric acid in the blood may result from the ingestion of purin bases even when no evidence of retention is found on examination of the urine. A number of experimental test meals given for the purpose of determination of whether or not the giving of purin-rich diets can increase the uric acid in the blood of healthy people shows that they cannot do so. It has been

⁸Vogt: *Deutsch. Arch. f. klin. Med.*, 1901, vol. lxxi, p. 21.

⁹Reach. *München. med. Wchnschr.*, 1902, vol. xlix, p. 215.

¹⁰Magnus-Levy: *Deutsch. med. Wchnschr.*, 1911, vol. xxvii, p. 778.

¹¹Pratt: *Am. Jour. Med. Sc.*, 1916, vol. cli, No. 1, p. 92.

clearly proved that the uric acid derived from exogenous purin does not accumulate in the blood unless there is a disturbance in the uric acid metabolism.

We have abundant analytical evidence to prove, therefore, that in gout there is increase in the uric acid concentration in blood without any increase in the other nonprotein nitrogenous constituents. Daniels and McCrudden are two observers who have reported several cases of gout in women without any increase in uric acid in the blood. No one else has found normal figures. On the contrary, Fine, who has contributed a great deal to the literature on uric acid values in gout and other conditions, states that he has never seen normal uric acid in blood in gout. It would seem that in the estimation of the amount of uric acid in the blood we have an excellent method of differentiating gout from rheumatism and other joint affairs. This is clearly evident. It must be remembered, however, that the increase in uric acid alone without any increase of urea nitrogen and creatinine, may occur in early chronic interstitial nephritis. In a recent communication, entitled: "The Relation of Gout to Nephritis as Shown by the Uric Acid in the Blood," Fine,¹² goes thoroughly into this question. He states that while uric acid concentrations up to 4 to 9 mgms. in blood are found in gout, these accumulations are not infallible signs of gout. Indeed, Garrod,¹³ von Jaksch,¹⁴ and von Noorden¹⁵ pointed this out in connection with the retention of uric acid as well as urea, but, of course, their observations were purely clinical. Owing to the fact that in early interstitial nephritis there is only an undue retention of uric acid in the blood, it is necessary to exclude this condition before adopting the diagnosis of gout. Fine states that there may be no undue accumulation of urea nitrogen and creatinine in early interstitial nephritis, uric acid values alone showing an abnormal figure over 2.5 mgms. He showed in collaboration with Myers and Lough¹⁶ very plainly that in early interstitial nephritis, there is first an accumulation of uric acid; secondly an accumulation of urea, and, finally, an ac-

¹²Fine: Jour. Am. Med. Assn., 1916, vol. lxvi, No. 26.

¹³Garrod, A. B.: Med. Clin., 1848, vol. xxxi, p. 83; and Treatise on Gout and Rheumatic Gout, 1876.

¹⁴von Jaksch: Zentralbl. f. inn. Med., 1896, vol. xvii, p. 545.

¹⁵von Noorden: Metabolism and Practical Medicine, 1907, vol. iii, p. 29; Ibid., 1914, vol. xvii, p. 487.

¹⁶Myers, Fine and Lough: Arch. Int. Med., 1916, pp. 570-583.

cumulation of creatinine in the blood. This is what these observers term their "stair-case" effect. Twelve cases came under their observation in which more than 10 mgms. of uric acid were found in the blood without any gouty symptoms. In one case as much as 27 mgms. were present. It was also observed by them that higher uric acid values were seen early in the cases than later, although during the agonal period there was a marked increase coincident with that of creatinine. Folin and Denis¹⁷ remarked on the fact that in the severest cases of uremia there was only a slight increase in the blood ammonia and that it was likewise only these cases in which a marked retention of creatinine occurred. They concluded from this that the human kidney removes the creatinine from the blood with remarkable ease and certainty. The completeness of the creatinine excretion, is, in fact, they further state, exceeded only by the still more complete removal of the ammonium salts.

Myers, Fine, and Lough¹⁸ give in tabular form some interesting data showing in a series of twenty-six cases studied, a decided increase in the concentration of the uric acid alone without any corresponding increase in urea nitrogen or creatinine. Some of these cases showed symptoms which in general are characteristic of early interstitial nephritis. In other cases, although the nephritis was not the predominant clinical condition, it would appear that the systemic disturbances resulting from, or associated with, a variety of conditions, such as tuberculosis, typhoid fever, pneumonia, carcinoma, cardiac disorders, chronic alcoholism, etc., are capable of exerting the same influence upon the kidney. It is not improbable that similar factors are at work in gout and the apparently uncomplicated cases of interstitial nephritis. These investigators also showed in tabular form four cases of diabetes with uric acid values of 10.5, 6.0, 5.0, and 7.6 mgms. respectively where there were similarly normal creatinine values, namely, 2.1, 2.0, 2.3 and 4.7 mgms., respectively. The last figure, of course, is an increase in creatinine. In this case the patient entered the hospital in coma and died several hours later; the urine contained very large amounts of albumin, acetone, and diacetic acid, and many granular casts. These observers point to the fact that their

¹⁷Folin and Denis: *Jour. Biol. Chem.*, 1914, vol. xvii, p. 487.

¹⁸Myers, Fine, and Lough: *Arch. Int. Med.*, 1916, pp. 570-583.

TABLE XII*

BLOOD PICTURES IN GOUT AND EARLY INTERSTITIAL NEPHRITIS**												
Date 1915- 1916	Case	Age	Sex†	Diagnosis	Uric Acid, Mgms. per 100 c.c.	Urea N, Mgms. per 100 c.c.	Creat- inine, Mgms. per 100 c.c.	Phthal- ein 2 Hrs. per cent.	Systolic Blood Pressure	Urine		
										Albumin	Casts	
9/ 3	M. K.	49	♀	Typical gout.....	9.5	13	1.1	48	230	+	—	
10/ 5	T. B.	57	♂		8.4	12	2.2	35	164	—	+	
3/24	H. L.	51	♂		7.8	12	2.9	59	120	—	—	
10/ 6	L. J.	43	♂		7.2	17	2.4	..	200	—	—	
10/ 6	C. P.	45	♂		6.8	14	1.7	..	..	..	..	
3/31	B. D.	25	♀	Miscellaneous cases Showing some evidence of early interstitial nephritis	7.7	20	2.6	45	168	+	—	
6/ 7	D. S.	56	♂		Slight edema.....	6.7	19	2.5	26	185	—	+
3/20	E. V.	14	♀		Hypertension.....	6.3	12	3.6	32	120	+	+
3/28	H. B.	55	♂		Endocarditis.....	6.1	16	2.8	57	120	—	+
3/23	H. J.	50	♂		Incipient nephritis..	6.0	18	2.9	36	140	+	+
3/14	G. C.	40	♀		Diabetes.....	5.5	15	2.1	46	147	—	+
3/22	M. S.	46	♀		Incipient nephritis..	5.0	12	2.5	52	190	+	+
				Gastritis.....								

*Pine: Jour. Am. Med. Assn., 1916, vol. lxvi, No. 26.

**Normal findings: Uric acid, from 1 to 3 mgms.; urea N, from 12 to 15 mgms.; creatinin, from 1 to 2.5 mgms.

†In this column, ♂ denotes male, and ♀ female.

series of thirty cases were apparently suffering with early interstitial nephritis, probably secondary in many instances to other systemic disturbances. They believe that an increased uric acid value alone without any increase in urea nitrogen or creatinine might serve as an aid to an early diagnosis. They also suggest that a retention of uric acid may be earlier evidence of renal impairment of an interstitial type than the classical tests of albuminuria and cylinduria. In Fine's later paper,¹⁹ he gives a table (see Table XII, page 199) of two groups of cases, the first composed of five cases giving the classical histories of gout, and the second consisting of seven cases with some evidence of incipient nephritis, such as slight albuminuria, cylinduria or diminished phenolsulphonphthalein output. These cases were given a purin-free diet several days before the examinations were made. The first two cases that he calls attention to gave typical histories of gout, but also showed one or more signs of nephritis and from this standpoint might well have been placed in the second group. He points to the striking similarity in the blood pictures in the two groups.

There is truly a slight increase of urea nitrogen and creatinine in group 2, but the increase is negligible. Fine states that many cases of gout have been reported²⁰ with blood uric acid concentrations as low or lower than the lowest in the above group 2. Fine propounds the following queries as a result of these figures: 1. Is gout merely a stage in the development of interstitial nephritis, whose further progress may be indefinitely delayed? 2. Is early interstitial nephritis merely potential gout, in which the clinical symptoms may or may not be eventually in evidence? 3. Is the uric acid retention of gout due to the specific condition, gout, or to a complicating early interstitial nephritis?

From these observations and reports we can readily recommend the advisability of blood chemical analyses in dealing with suspected cases of gout, rheumatic fever, and early interstitial nephritis. No adequate comprehension of cases of this kind can be obtained by mere urinary findings or the best clinical symptoms.

¹⁹Fine: *Jour. Am. Med. Assn.*, 1916, vol. lxvi, No. 26.

²⁰Folin and Denis: *Jour. Biol. Chem.*, 1913, vol. xiv, p. 40; and *Arch. Int. Med.*, 1915, vol. xvi, p. 35.

CHAPTER XXVIII.

BLOOD CHEMISTRY AND NEPHRITIS.

It has already been noted that in gout we have an alteration in the concentration of one of the nonprotein nitrogenous blood constituents; namely, uric acid. Attention has also been called to the fact that in early interstitial nephritis we have likewise only an accumulation of uric acid. It will be necessary in discussing the blood figures in chronic nephritis, interstitial or parenchymatous in variety, to refer to some of the facts of nitrogenous metabolism. Nonprotein blood constituents are urea nitrogen, uric acid, creatinine, creatine, sugar, chlorides in the form of sodium chloride, and cholesterol. The normal amounts of these constituents are as follows:

Nonprotein nitrogen	25 to 30 mgms. per 100 c.c. blood
Urea nitrogen	12 " 15 " " " " "
Uric acid	1 " 3.0 " " " " "
Creatinine	1 " 2.5 " " " " "
Creatine	5 " 10 " " " " "
Sugar	0.08-0.12%
Chlorides as sodium chloride	0.65%
Cholesterol	0.15%

For purposes of comparison we refer to the table showing values in various diseases (Fig. 63, page 202) in which we tabulate the normal findings and the changes met with in the common diseases. We would also refer the reader to Fig. 64, page 203, showing nonprotein nitrogen, etc., in which more elaborate figures are shown.

At this point we wish to refer to the significance of nonnitrogenous metabolism:

Total Nitrogen is eliminated in the proportion of 15 grams per diem. It leaves the body as follows:

Urea (grams)	25	(12 gm. N)	or 85%
Creatinine	1.5		or 5%
Uric acid	0.5		or 2%
Ammonia	0.5		or 4%
Rest nitrogen	0.5		or 5%

DISEASE	UREA N		URIC ACID		CREATININE		SUGAR	
	MGMS. PER 100 ^{cc} of BLOOD		PER 100 ^{cc} of BLOOD		PER CENT		PER CENT	
NORMAL	12-15	1-3	1-2.5	0.08-0.12				
GOUT		35-6						
MILD DIABETES				0.15-0.30				
SEVERE DIABETES				0.30-1.10				
CHRONIC NEPHRITIS	15-50	1-4	1-3					
UREMIC NEPHRITIS	80-300	4-15	4-34	0.10-0.20				

Fig. 63.

THE CHARACTERISTIC BLOOD PICTURES in GOUT, DIABETES & NEPHRITIS.

DISEASE	TOTAL SOLIDS	TOTAL NITROGEN	NON PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	CREATININE	SUGAR	CHLORIDES AS SODIUM CHLORIDE	CHOLESTEROL
	PER CENT	MGMS. PER 100 CC. BLOOD							PER CENT
NORMAL	20.0	3.00	25-30	12-15	1-3	1-2.5	5-10	0.08-0.12	0.65 0.15
GOUT					35.6				
MILD DIABETES							0.15-0.30		
SEVERE DIABETES	17-20	18-29					0.30-1.10	0.57-0.61	0.15-0.30
CHRONIC NEPHRITIS	13-19		30-80	15-50	1-4	1-3		0.54-0.75	0.17-0.35
UREMIC NEPHRITIS	12-18	17-27	120-350	80-300	4-15	4-34	5-31	0.10-0.20	0.48-0.64
									0.17-0.35

Fig. 64.

Where does urea come from? In digestion protein matter is broken down into amino-acids which are picked up by the blood just as pieces of metal are picked up by a magnet. Some of the amino-acids are retained and others are transformed into ammonia and eliminated. The greater part of the nitrogen that is eliminated is exogenous (coming from food) and its elimination occurs in the form of urea. The blood holds up the carbonates and preserves its neutrality by this means, by eliminating or getting rid of the acids. The greater part of the acids in urine are made up of acid phosphates, derived from the blood. When the blood is no longer able to get rid of its acids, it calls upon its ammonia for help. This has already been alluded to in the chapter on acidosis (see page 170). The determining factor in respect to nitrogen in urine is the neutrality of the blood. If you administer enough alkali, you can cause the nitrogen to entirely disappear. It is a well-known fact that rabbits eliminate no nitrogen in their urine because they live on a diet that contains a good deal of carbonates. Nitrogen depends upon the hydrogen-ion concentration of bodily tissues.

The source of creatinine is entirely endogenous. It is constant day by day in the body.

There have been some interesting data experimentally obtained as to the effect of the administration of creatine and creatinine to animals. Folin¹ was the first by means of his colorimetric methods to show that the quantitative conversion of creatine or creatinine to creatine *in vitro* was far more difficult than previous statements would lead one to believe. He was unable to prove that feeding experiments with creatine in man were followed by conversion into creatinine. Other experimental observations were made by Klercker,² Wolf and Shaffer,³ van Hoogenhuyze and Verploegh,⁴ and others. Myers and Fine⁵ conclude from their experimental observations that the administration of creatinine appears to exert a slight increase on the muscle content of creatine. When creatinine was administered an average of 80 per cent appeared in the urine but no elimination of crea-

¹Folin: Hammarsten's Festschrift, 1906, vol. iii.

²Klercker: Beitr. z. Phys. u. Path., 1906, vol. viii, p. 59; Biochem. Ztschr., 1907, vol. iii, p. 45.

³Wolf and Shaffer: Jour. Biol. Chem., 1908, vol. iv, p. 489.

⁴van Hoogenhuyze and Verploegh: Ztschr. f. phys. Chem., 1908, vol. lvii, p. 161.

⁵Myers and Fine: Jour. Biol. Chem., 1913, vol. xvi, p. 169.

tine was detected. Folin and Denis⁶ experimentally failed to show any creatinine formation from the administration of creatine, although they noted a slight accumulation of creatinine in the blood and a slight diminution in the muscle. In a later paper Myers and Fine⁷ reiterate their belief in the uniformity obtained from the creatine content of the muscle of certain animals, particularly the rabbit, and suggest that this might ultimately be found to be the underlying factor in the constancy in the excretion of creatinine. Their results have been confirmed by Dorner,⁸ Mellanby,⁹ Riesser,¹⁰ Palladin and Wallenburger,¹¹ and Baumann.¹²

It appears to be fairly well established, therefore, that creatinine resides in muscle and that it is constantly present in blood in about the same quantity at all times in health in the adult. The importance of creatinine in routine blood chemical analysis in connection with chronic nephritis has also been very well established. It seems strange that for so long a time only estimations of total nonprotein nitrogenous blood constituents were the order of the day. At the present time there is no one ingredient that is more important to estimate than is creatinine. Cases of blood retention of which uremia constitutes the most striking type, show accumulation of creatinine as well as urea nitrogen and uric acid.

Shaffer has shown that it is constant hour by hour. It is not materially increased by protein food intake. It is always present in muscle tissue, as shown by Shaffer,¹³ and Myers and Fine.¹⁴ Myers and Fine¹⁵ believe that the urinary creatinine is originated from muscle tissue. These authorities¹⁶ have published a number of observations on the metabolism of creatine and creatinine. Their paper on "The Presence of Creatinine in Muscle" shows the content of creatinine in fresh muscle in quantities varying

⁶Folin and Denis: Jour. Biol. Chem., 1914, vol. xvii, p. 493.

⁷Myers and Fine: Jour. Biol. Chem., 1915, vol. xxi, p. 289.

⁸Dorner: Ztschr. f. phys. Chem., 1907, vol. lii, p. 259.

⁹Mellanby: Jour. Physiol., 1907-8, vol. xxxvi, p. 447.

¹⁰Riesser: Ztschr. f. phys. Chem., vol. lxxxvi, p. 444.

¹¹Palladin and Wallenburger: Compt. rend. Soc. de biol., 1915, vol. lxxviii, p. 111.

¹²Baumann: Jour. Biol. Chem., 1914, vol. xvii, p. 15.

¹³Shaffer: Am. Jour. Physiol., 1908-9, vol. xxiii, p. 4.

¹⁴Myers and Fine: Am. Jour. Med. Sc., 1910, vol. cxxxix, p. 256.

¹⁵Myers and Fine: Jour. Biol. Chem., 1913, vol. xiv, p. 24.

¹⁶Myers and Fine: Jour. Biol. Chem., 1913, vol. xv, p. 304; Ibid., 1913-14, vol. xvi, p. 174; Ibid., 1914, vol. xvii, p. 65; Proc. Soc. Exper. Biol. and Med., 1913, vol. xi, p. 15; Ibid., 1915, vol. xxi, No. 2, p. 383.

in the cases of rabbits from 4.5 to 5.9 mgms., 5.7 in human muscle in leg amputated for sarcoma, 2.6 in leg amputated for gangrene, 6.8 in pectoral muscle of case of interstitial nephritis, 6.8 in heart muscle from uremic case, 18.1 in psoas muscle in interstitial nephritis. They showed,¹⁷ as did Shaffer,¹⁸ and Folin and Denis,¹⁹ that the quantity of creatinine present in the muscle is much greater than that of the blood, liver or any other tissue. The fact that the greater portion of the preformed creatinine present in the body is found in the muscle, strongly suggests that this is the chief creatinine-forming tissue.

Uric acid is partly exogenous and partly endogenous; partly from the metabolism of food and partly from that of our own tissues. This is about half and half. If liver is eaten, we can raise

the amount of uric acid present. It comes from purin, then changed to xanthin, and then to uric acid. It has to be de-aminoized before change to xanthin takes place. This takes place by hypoxanthin being formed from adenin, and xanthin is formed from guanin.

The following graphic representation shows this:

The second part of the process is an oxidation, i. e., the conversion of hypoxanthin into xanthin and this conversion into uric acid. Uric acid, therefore, is the chief end-product in man of nucleo-protein metabolism.

Uric acid is a difficult substance to dissolve. It is soluble 1 part

¹⁷Myers and Fine: Jour. Biol. Chem., 1915, vol. xxi, p. 389.

¹⁸Shaffer: Jour. Biol. Chem., 1910, vol. vii, pp. 23, 30.

¹⁹Folin and Denis: Jour. Biol. Chem., 1914, vol. xvii, p. 501.

in 39 of pure water. Urates are soluble in 1 part in 500 under conditions as they exist in the body. Uric acid is the most difficult for the kidney to excrete of the nonprotein blood constituents; urea comes next, and creatinine last. Expressed in other terms, creatinine is the easiest constituent for the kidneys to eliminate, urea is the next, and uric acid is the last to be eliminated. Again, urea exists in the body in twenty times as much concentration as creatinine and it therefore takes twenty times as much work for the kidney to eliminate its urea as its creatinine.

With these fundamental facts before us, let us consider what has been done in the past with respect to studying from a diagnostic standpoint the character of nonprotein metabolism in disease. It might be mentioned in passing that the estimation of the kidney function has long been considered a favorite method of determination of metabolic faults. For instance, the indigo-carmin test, the phlorizin test and cryosecopy of blood and urine each have had their vogue and have been practically abandoned because of the meager information obtainable thereby. Possibly Geraghty and Rowntree,²⁰ with their phenolsulphonphthalein test, did more to advance the cause of kidney functional tests than any of their predecessors. This test of kidney function is quite reliable but it has its limitations. It is an excellent method of estimating the function of the kidney for the moment but does not represent the condition of the kidneys so far as retention of objectionable constituents are concerned, over a long enough period of time to accurately weigh bodily metabolic changes in nonprotein nitrogen.

The question of the comparative value of the Geraghty and Rowntree test and the blood chemical analysis for nonprotein nitrogenous constituents was experimentally carried out by Frothingham, Fitz, Folin, and Denis.²¹ Rabbits were used and experimental nephritis produced by the injection of uranium nitrate (1.25 to 3 mgms.) subcutaneously. The first series of animals were killed under anesthesia by bleeding from the carotid arteries. They were killed on consecutive days from one to ten days after administration of uranium nitrate. In a second series

²⁰Geraghty and Rowntree: *Jour. Pharm. and Exper. Therap.*, 1910, vol. i, p. 579.

²¹Frothingham, Fitz, Folin and Denis: *Arch. Int. Med.*, 1913, vol. xii, p. 145.

of experiments the animals were allowed to recover, and the blood chemical analyses and the phenolsulphonphthalein tests were made periodically. The blood specimens for chemical analyses were taken from the marginal ear veins. The rabbits were kept in cages, fed on carrots and hay, 100 grams of carrots per day, with 50 c.c. of water administered by means of a stomach tube before the injection of the phenolsulphonphthalein (1 c.c. containing 6 mgms.) into the muscles of the thigh. The animals were kept in a small cage over a glass funnel to prevent loss of urine. After 70 minutes the urine was obtained by massage. The determination was made according to Geraghty and Rown-tree's method (see page 89). It was seen that the normal rabbits have about 30 mgms. of urea nitrogen per 100 c.c. The rate of phenolsulphonphthalein in excretion in normal rabbits is about 60 per cent in 70 minutes.

These experimental observations on uranium nephritic rabbits showed a decrease in the excretion of phenolsulphonphthalein and a great accumulation of nonprotein nitrogenous constituents. The decrease in the phenolsulphonphthalein amounted to as little as a trace only. The retention of nonprotein nitrogen amounted to as much as 216 mgms. and of ureas as much as 172 mgms. The retention of nitrogen remained high even where the phenolsulphonphthalein began to improve. In general the tests paralleled each other. In another series of experiments, the blood was collected every two or three days from the veins. The nitrogen seemed to go on being retained even where the phenolsulphonphthalein excretion was improving. This seemed to prove that the nitrogenous retention represented the difference between that *eliminated* and that *produced*, whereas the phenolsulphonphthalein is an indicator of *elimination alone*. This represents essentially the difference between the two tests. The percentage of phenolsulphonphthalein excreted affords an index of the kidney function at the time the test is made. The result is apparently not at all influenced by the length of time the kidney may have been in the condition indicated by the test. In general these tests parallel each other as indicators of kidney function with the essential difference, however, that the amount of phenolsulphonphthalein excretion shows the renal function for the moment. The

amount of nonprotein nitrogen and urea nitrogen in the blood is rather a measure of accumulating difference between the waste nitrogen produced in metabolism and amount eliminated by the kidneys. The time element, the duration of the condition, is therefore an important factor in weighing up to these results. The outcome of a case cannot be estimated nearly so well by functional kidney tests as by blood chemical analyses. Foster²² reported a case of marked kidney disease with normal elimination of phenolsulphonphthalein. If the prognosis had been based upon the phenolsulphonphthalein output, this patient would have recovered, but, as a matter of fact, he died. Again, he mentions the fact that a low output would not indicate a fatal termination in cases of chronic nephritis. In Foster's case with an output of 28 the patient died within two days in coma. It can thus be seen that a normal output of phenolsulphonphthalein does not necessarily indicate kidney lack of function insofar as nonprotein nitrogenous retention is concerned, nor does a lower output than normal indicate the outcome of a case. It will be seen later that we have in the estimation of the creatinine values particularly, a very valuable means of prognosis.

Assuming, therefore, that the moment is now at hand in diagnosis, where we must weigh up the character of blood retention in cases of nephritis, it is manifest that the blood chemical figures are the most trustworthy that can be gathered. We have noted already the percentage of nonprotein nitrogenous concentrations in health. In degenerative conditions of the kidney, these blood constituents are markedly altered. In early interstitial nephritis, we have the beginning of retention in the shape of an accumulation of but one ingredient, namely, uric acid. Here values may be seen as high as from 4 to 6 mgms. per 10 c.c. of blood, as opposed to the normal values of 1 to 3.0 mgms. Next we have in more advanced cases an accumulation of creatinine in the blood, the figure 2.5 mgms. representing the upper limit of the normal and any figure over this constituting an abnormality. This accumulation of the three constituents in their order, uric acid first, urea second and creatinine third, represents the fact already detailed, that uric acid is the most difficult substance for the kidney to ex-

²²Foster, N. B.: Arch. Int. Med., 1913, vol. xii, p. 452.

crete; urea occupying an intermediate position, while creatinine is the easiest. We have alluded before to the "stair-case" effect of retention first pointed out by Myers and Fine. Chace and Myers²³ give a tabulated list of cases showing this effect (see Table XIII, page 211).

It can readily be seen from this table that the first accumulation in the blood when kidney function is interfered with by beginning chronic interstitial nephritis is in the uric acid values, next there occurs an accumulation of urea as well as uric acid, and finally, in uremic nephritis we have an accumulation of uric acid, urea nitrogen, and creatinine. This seems particularly interesting and important in view of the fact that the urinary changes in some of these cases are exceedingly scant. The finding of albumin and casts is often made, but this gives the clinician but little information as regards the true metabolic processes that are going on and the exact state of kidney function. We cannot well understand how a clinician can safely pass judgment in a case of chronic nephritis without an examination of the blood for these ingredients.

To recapitulate, we know that the greatest amount of retention of urea, uric acid, and creatinine occurs in chronic interstitial nephritis particularly when uremia is at hand. A prognostic sign of no mean importance is that first pointed out by Myers and Lough²⁴ in their paper on "Diagnostic Value of Creatinine in the Blood in Nephritis." They showed at that time (1915) that when creatinine in the blood appeared in the concentration of 5 mgms. per 100 c.c. of blood and over, that every one of these cases terminated fatally. Of the eleven cases in their series showing over 5 mgms. of creatinine per 100 c.c. of blood, all terminated fatally in from a few days to two months. In this group of cases the phenolsulphonphthalein output was practically zero, with but one exception. These cases of creatinine values of 5 mgms. or above were: a case of mercuric bichloride poisoning, with creatinine value of 33.3 mgms.; a case of chronic interstitial nephritis in uremia with creatinine of 20.5 mgms.; six other cases of interstitial nephritis, with creatinine values of 20.0,

²³Chace and Myers: Jour. Am. Med. Assn., 1916, vol. lxvii, No. 13, p. 929.

²⁴Myers and Lough: Arch. Int. Med., 1915, vol. xvi, pp. 536-546.

TABLE XIII*

URIC ACID, UREA NITROGEN, AND CREATININE OF BLOOD IN INTERSTITIAL NEPHRITIS**

Date, 1915- 1916	Case	Age	Sex†	Diagnosis	Condition	Mgms. per 100 c.c. of Blood			Phthal- ein 2 Hrs., per cent.	Systolic Blood Pres- sure	Urine	
						Uric Acid	Urea N	Creat- inine			Albu- min	Cast- s
I												
9/17	H. L.	23	♂	Pulmonary tuberculosis.....	Unchanged	6.5	16	2.7	58	130	++	+
8/10	E. H.	41	♂	Pericarditis.....	Unchanged	5.6	13	2.1	45	150	—	—
10/12	F. D.	45	♂	Interstitial nephritis.....	Unchanged	5.5	12	2.5	37	185	—	+
3/ 6	B. D.	35	♀	Diffuse nephritis.....	Unchanged	9.6	19	2.4	45	175	+	+
II												
8/11	J. J.	65	♂	Early interstitial nephritis.....	Unchanged	9.5	25	2.5	13	185	+	+
7/21	D. S.	56	♂	Early interstitial nephritis.....	Unchanged	6.6	24	3.3	26	185	—	+
9/21	D. D.	52	♂	Early interstitial nephritis.....	Unchanged	8.7	20	3.6	20	100	+	+
8/ 3	C. M.	54	♂	Early interstitial nephritis.....	Unchanged	6.3	31	2.0	23	150	—	—
III												
1/ 6 }	L. P.	57	♂	Moderately severe chronic inter- stitial nephritis.....	Improved..	8.0	80	4.8	0	240	++	++
3/ 1 }						4.9	17	2.9	10	170		
4/23 }	J. P.	34	♂	Moderately severe chronic dif- fuse nephritis.....	Improved..	8.3	72	3.2	25	238		
5/21 }						5.3	21	1.9	43	145		
1/15 }	W. C.	49	♂	Moderately severe chronic dif- fuse nephritis.....	Improved..	9.5	44	3.5	38	210	++	++
1/25 }						2.5	19	1.9	52	120	++	++
IV												
4/11	E. C.	50	♀	Typical fatal case of chronic in- terstitial nephritis.....	Died.....	22.4	236	16.7	0	210	++	Pus
3/23	T. D.	34	♂	Typical fatal case of chronic in- terstitial nephritis.....	Died.....	15.0	240	20.5	2-3	225	++	+
1/25	S. H.	37	♂	Typical fatal case of chronic in- terstitial nephritis.....	Died.....	14.3	263	22.2	0	220	++	+
4/15	J. W.	34	♂	Typical fatal case of chronic in- terstitial nephritis.....	Died.....	8.7	144	11.0	Trace	225	+	+

* Chase and Myers: Jour. Am. Med. Assn., 1916, vol. lxvii, No. 13, p. 929.

** Normal findings: uric acid from 2 to 3 mg.; urea nitrogen, from 12 to 15 mgm.; creatinine, from 1 to 2.5 mgm. per 100 c.c.

† The symbol ♂ signifies male; ♀ signifies female.

16.7, 16.6, 14.7, 11.0 and 5.3 mgms. respectively; three cases of chronic diffuse nephritis, with uremia, with creatinine values of 14.7, 7.4, and 7.0 mgms. of creatinine respectively. They have three times as many cases on record in which this fact was borne out.

The prognostic value of the finding of 5 mgms. of creatinine or over has been confirmed by the writers, together with Schisler, in a group of cases of thermic fever recently studied at the St. Louis City Hospital, a full report of which will be shortly published. Here we had a set of blood findings identical in all particulars with those of uremia. We present in Fig. 65 a tabulated picture of these cases, showing their blood and urinary findings.

We are able to record three cases of thermic fever in which the creatinine values of 4.8, 5.0, and 6.1 mgms. respectively, pointed to a fatal ending, which ensued within forty-eight hours from the time when the record was made. In the case of O'Conner, the observation was made on August 1, and the patient died the same day. He showed urea nitrogen of 33 mgms., uric acid 13.2, creatinine 4.8 (slightly below the fatal prognostic point), and blood sugar 0.15%. His urine analysis showed albumin and coarsely granular casts. The next case (Fischer) ran rather a long course for a case of thermic fever which was from the outset quite severe. This individual entered the hospital on August 2, 1916, showing a severe picture, semiconscious, rise in temperature to 108° F., delirium. His blood findings on the first day were urea nitrogen 32, uric acid 8.6, and creatinine 4.1 mgms. From day to day he was tested and showed at first a slight decline in his blood findings. On the eighth day of his stay in the hospital his creatinine reached the fatal point of 5.0 mgms. He died two days later. Autopsy on this case showed simply cloudy swelling of the kidneys and no other gross changes anywhere. It might be mentioned that his Wassermann of blood and spinal fluid was negative. His urinary findings during all this time showed at first a very heavy amount of albumin and moderate number of granular casts. Towards the end of life the urine cleared up as regards albumin, but, on the day before death, the microscopical picture showed the fields actually crowded with granular casts. The next two cases (Huth and Ship) are especi-

BLOOD AND URINE FINDINGS IN THERMIC FEVER

BLOOD ANALYSIS										URINE ANALYSIS★									
CASE	DATE	OUTCOME	UREA NITROGEN PER 100CC.	MGMS. PER 100CC.	CREATININE PER CCM	SUGAR	REMARKS		SPECIFIC GRAVITY	ALBUMIN	SUGAR	ACETONE	DIACETIC ACID	INDICAN	MICROSCOPICAL EXAMINATION	REMARKS			
O'Conner	8/1	DIED	33	132	480	150	RETENTION HIGH. PATIENT DIED SAME DAY.		1015	#	NEG	NEG	NEG	###	Moderate number of coarsely granular casts and red blood cells. Occasional leucocytes.	FINDINGS SERIOUS			
	8/2		32	86	41	1082	High retention indicating probable fatal outcome. Patient died two days after creatinine reached 5.0 mgms. per 100 cc.			###	NEG	#	#	#	Moderate number of granular casts and epithelial cells.	Urinary findings indicated some marked renal disturbance, but not same importance as blood findings.			
Fischer	8/3		39	98	456	1065													
	8/4	DIED	39	79	447	1080													
	8/8		45	71	394	1056													
	8/9		44	68	24	010													
	8/10		55	69	50	0174													
Huth	8/12		89	82	50	020													
	8/2	RECOVERED	26	96	383	068	Retention not high. Patient recovered although clinical signs seemed bad.			NEG	NEG	NEG	NEG	###	Very many granular casts and occasional leucocytes. Such number of casts is rarely seen.	Remarkable number of casts on last day of life.			
Ship	8/12	RECOVERED	14	33	20	020				#	NEG	+	+	#	Moderate number of coarsely granular casts and occasional leucocytes. Very occasional leucocyte.				
	8/3	DIED	76	148	61	007	Clinical signs good. Retention high, attracting attention to fatal prognosis. Died one day later.			Very faint Trace	NEG	NEG	NEG	NEG	#	Moderate number of epithelial cells. Very occasional red blood cells. Two finely granular casts found after a prolonged search.			
Muich	8/4	RECOVERED	19	33	30	038	Observation made when convalescent.												

★ Very large amount
Moderate amount
+ Small amount.

Fig. 65.

ally interesting in that the one case (Huth), with an apparently hopeless clinical symptomatology, had a very good blood picture (urea nitrogen 26, uric acid 9.6, creatinine 3.83 mgms.) while the other case (Ship), observed at the same time, with a much more favorable clinical picture, showed a very grave set of blood findings; viz., urea nitrogen 76, uric acid 14.8, and creatinine 6.1 mgms. In the Huth case an unfavorable clinical prognosis was made, but a good prognosis was issued after the blood examination was completed. True to the latter prediction, he promptly recovered. The second case with a rather favorable clinical prognosis was condemned by the blood findings of creatinine over 5 mgms. True to this prediction, he died on the following morning. Both cases showed substantially the same urinary findings, thus illustrating that no prognostic record could accurately be made in this way. The last case was observed and tested during the period of his convalescence and showed almost normal findings.

These cases, therefore, served to illustrate the great value of blood chemical methods in first demonstrating that the condition met with in thermic fever is quite analogous to that seen in uremic nephritis, secondly, in proving Myers, Lough and Chace's contention that the finding of over 5.0 mgms. of creatinine in blood serves to indicate a fatal ending in any case. A report of a most unusual case of chronic interstitial nephritis, with findings in blood and urine made by Halsey,²⁵ serves as an object lesson in pointing out the value of this type of work. This patient was well enough to visit the observer's office with symptoms of a subjective nature so slight as to be almost incompatible with the findings on physical examination and blood analyses and subsequent, rapid, fatal ending. He was on his way to Florida, but stopped off in New York with but little idea evidently of the seriousness of his condition. His urine showed no albumin or casts. His blood examination showed urea nitrogen 97, uric acid 6.6, creatinine 17.5, blood sugar 0.18 per cent, blood plasma combining power 50. Because of these desperate findings he was further detained and carefully observed. After three days of nitrogen-poor diet, the blood examination showed urea nitrogen 129 mgms., uric acid 6.3, creatinine 21.8, blood sugar 0.18 per cent. His nitrogen in-

²⁵Halsey, R. H.: Jour. Am. Med. Assn., June 10, 1916, vol. lxvi, No. 24, p. 1847.

take was restricted, and seven days later his findings were: urea nitrogen 132, uric acid 7, and creatinine 22.3, with an increase in the carbon dioxide combining power of his blood plasma to 53. Four days later, still on nitrogen-poor diet, he showed urea nitrogen 144, uric acid 6.1, and creatinine 28.9. His carbon dioxide combining power was diminished to 50. His protein diet was here increased owing to the effect on the tissues of too long an abstinence from nitrogenous food. Three days later the findings were urea nitrogen 150, uric acid 5.6, creatinine 24.2, blood sugar 0.20 per cent and carbon dioxide down to 33. Further blood examinations showed a corresponding rise in blood constituents and death of patient occurred on the twenty-fifth day of his observation. This patient showed physically a picture of hypertension with but the slightest hypertrophy of the heart. The conclusions of Halsey from this record were very aptly stated; i. e., that with the examination of the urine only, the seriousness of the patient's condition would not have been discovered, also that while the phenolsulphonphthalein test was of value in indicating the status of the patient for the moment, the amount of urea and creatinine, particularly the latter, gave the best clue as to the progress and the prognosis.

Another set of conditions in which the blood chemical analysis should prove of striking value to the clinician is the group of cases called cardio-vascular with only secondary renal disturbance. Differentiation of these cases from cases of true nephritis with secondary cardiac and blood vessel change might well be made by means of the colorimetric methods. Through the courtesy of Dr. Edwin Schisler of the St. Louis City Hospital Staff, we are permitted to record some data on this group of cases (see Table XIV).

It will be readily seen that in these cases which showed the symptomatology of mixed cardiac and renal disease, there was little if any retention of the nonprotein nitrogenous ingredients in blood. The importance of blood chemical analyses in this variety of clinical condition can well be appreciated.

Test Meal for Renal Function and Ambard Coefficient.

Besides the well known phenolsulphonphthalein functional kid-

TABLE XIV

NAME	DATE	SEX*	UREA NITROGEN	URIC ACID	CREATININE
			Mgms. per 100 c.c. of Blood	Mgms. per 100 c.c. of Blood	Mgms. per 100 c.c. of Blood
"D"	7/28/16	♂	13	3.2	2.7
"B"	8/1/16	♂	12	2.8	2.8
"S"	8/2/16	♂	12	1.0	2.8
"M"	8/10/16	♂	12	2.4	2.7

* ♂ Male.

♂ Female.

ney test and the estimation of urea nitrogen, uric acid, creatinine, and sugar in blood, there are other measures of estimation of bodily metabolism as respect kidney function. A work of this kind would be incomplete if these were omitted. The other two methods which are used for certain definite reasons are those known as the Ambard coefficient of urea excretion, and the test meal for renal function.

The renal test meal and the estimation of renal function by this means is exceedingly simple in hospital practice but difficult to carry out in private practice. The urine is collected every two hours during the day, while the patient is on a full diet, and a ten to twelve hour specimen is collected at night. No food or drink is taken except at meal times. The collection of the night specimen is begun three hours after the evening meal. A normal test yields a maximum specific gravity of 1018 or more. The specific gravity varies but nine points or more from the highest to the lowest figure, and the night urine is small in amount, 400 c.c. or less and of high specific gravity, 1018 or over. A lowering of the maximum specific gravity, a fixation of the specific gravity and a nocturnal polyuria are the signs indicative of diminished renal function.

Mosenthal and Lewis²⁶ have given us an excellent account of these two measures as compared to the Geraghty and Rowntree test and the estimation of the nonprotein nitrogenous constituents in blood. They insist upon regarding each one of these measures as particularly designed to cover certain characteristics of each

²⁶Mosenthal and Lewis: Jour. Am. Med. Assn., Sept. 23, 1916, vol. lxxvii, No. 113, p. 933.

case and speak of them seriatim. Each has its place, each its indication, and from each valuable deductions may be drawn. The Ambard coefficient of urea excretion expresses numerically the relation between the concentration of urea in blood and the rate of excretion of urea in the urine. As a result of the study of normal human beings, Ambard²⁷ has asserted that when the concentration of urea in the urine is constant, the quantity of urea excreted in the urine varies proportionately to the square root of the concentration of urea in the blood; thus:

$$\frac{\text{Urea in blood}}{\text{Rate of excretion}} = \text{Constant, or } \frac{\text{Urea in blood}}{\sqrt{\text{Excretion per unit of time}}} = \text{Constant}$$

Also, when the concentration of urea in the blood remains constant, the quantity excreted in the urine varies inversely as the square root of the concentration in the urine; thus:

$$\frac{\text{Rate of excretion I}}{\text{Rate of excretion II}} = \frac{\sqrt{\text{Concentration II}}}{\sqrt{\text{Concentration I}}}$$

Or, as expressed by Mosenthal and Lewis:

$$K = \frac{Ur}{\sqrt{D \times \frac{70}{P} \times \frac{C}{25}}}$$

In which K = the coefficient of urea excretion.

Ur = urea grams per liter of blood.

D = urea grams excreted in urine in 24 hours.

C = urea grams per liter of urine.

P = body weight in kilograms.

70 = standard body weight in kilograms.

25 = standard concentration of urea grams per liter of urine.

McLean and Selling²⁸ have controlled Ambard's original method by using the exact methods of Folin, and state that "Ambard's coefficient, when computed from results obtained by the accurate methods of Folin and his collaborators, varies in normal persons only between comparatively narrow limits, and may be regarded as constant," and further "that ingestion of urea does not materially alter the value of Ambard's coefficient, provided sufficient

²⁷Ambard: *Physiologie normale et pathologique des reins*, Paris, 1914.

²⁸McLean and Selling: *Jour. Biol. Chem.*, 1914, vol. xix, p. 31.

time is allowed for absorption before examination is made. The normal coefficient is between 0.06 and 0.09, 0.08 being considered the figure." Quoting from Mosenthal and Lewis:²⁹ "When the values rise above 0.09, some impairment of the power of the kidney to excrete urea is indicated. Inability of the kidney to eliminate urea in proportion to the concentration of the blood urea results in an increase in proportion to the concentration of the blood urea results in an increase in Ambard's coefficient. In a normal individual it will remain within the limits mentioned, no matter what the height of blood urea; in cases with impaired renal function, however, the kidney does not answer the diuretic stimulus of the blood urea adequately, too little urea is put out, and the result is a rising coefficient, whether the urea in the blood be high or low. The degree of the impairment of renal function, as indicated by the various levels of Ambard's coefficient, is indicated in Table XV.

"The test meal for renal function which Mosenthal and Lewis refer to consists in the two hour collections of urinary specimens during the day, while the patient is on a full diet, and of a ten to twelve hour specimen at night. The patient is given no food or fluid except at meal times. The collection of the night specimen is begun three hours after the evening meal. Under these circumstances, a normal test yields a maximum specific gravity of 1018 or more, the specific gravity varies 9 points or more from the highest to the lowest, and the night urine is small in amount (400 c.c. or less) and of a high specific gravity (1018 or more). These criteria are the same as those originally demanded of a normal test, with the exception that a difference of 9 degrees between the highest and the lowest observations has been called normal, instead of 10. A lowering of the maximal specific gravity, a fixation of the specific gravity and a nocturnal polyuria are the signs indicating a diminished renal function.

"Table XV gives the various degrees of impairment as indicated by the test meal for renal function, as compared with the other tests. The salt, nitrogen, and other urinary constituents may be determined in these specimens, and valuable information may be obtained as to the ability of the body to excrete these

²⁹Mosenthal and Lewis: Jour. Am. Med. Assn., Sept. 23, 1916, vol. lxxvii, No. 113, p. 933.

TABLE XV

SCALE OF DEGREE OF IMPAIRMENT OF RENAL FUNCTION AS INDICATED BY THE TESTS EMPLOYED									
"Degree of Impairment" of Renal Function	Phenol- sulphon- phthalein per cent.	Nonprotein N of the Blood, Mgms. per 100 c.c.	Urea N of the Blood, Mgms. per 100 c.c.	Ambard's Coefficient of Urea Excre- tion	Test Meal for Renal Function				
					Night Urine		Variations in Sp. Gr. When the Highest Sp. Gr. is:		
					C.C.	Sp.Gr.	18	17-15	14 and 13
Normal.....	0	30—	15—	0.090—	400—	18+	9+		12—
Slight.....	+	31-45	16-27	0.091-0.115	401-600	16 and 17	8-5	6+	
Moderate.....	++	46-65	28-44	0.116-0.220	601+	15—	4—	5 and 4	
Marked.....	+++	66-90	45-64	0.221-0.350				3—	6+
Maximal.....	++++	91+	65+	0.351+					5—

After Mosenthal and Lewis.

substances. However, the simple procedure of measuring the volume of the urine and determining the specific gravity yields sufficient data to give an adequate idea of renal function in many respects, and the quantitative chemical determinations may be resorted to when more detailed information is desired. In order to study the relation to one another of the evidences of impaired renal function obtained by these various tests, a somewhat arbitrary scale of four degrees of impairment; slight, moderate, marked, and maximal, was determined on. The exact figures which the majority of experienced observers consider as indicating normal function, and these various degrees of subnormal function, were selected and the findings in over 200 patients were grouped in accordance with this scale."

The contention of Mosenthal and Lewis is that each one of these methods calls attention to a relative degree of involvement of kidney function and that each one of them has a significance apart from the others. They conclude, therefore, that a comparison according to this method is an extremely valuable aid in the treatment and prognosis of diseases of the kidney. They correctly assert that so far as nonprotein nitrogenous retention is concerned, differentiation must be made in weighing the results in the balance between kidney efficiency, diet and protein destruction. It must be remembered, however, that the chemical analysis of blood offers perhaps the readiest method and the most significant in its findings over all other methods alluded to above. We are, therefore, inclined to believe that the renal test meal, although of exceedingly great utility, cannot approach in definiteness the blood chemical tests. So far as the estimation of Ambard's coefficient is concerned, we are inclined to agree with Chace and Myers³⁰ that this method gives no additional information over the estimation of uric acid, urea and creatinine of the blood, and the phenolsulphonphthalein of the urine. This is in line with the conclusions of Addis and Watanabe,³¹ that the rate of urea excretion in man varies under physiological conditions in a manner which cannot be explained by the concentrations of urea in the blood and urine.

The value of the Ambard quotient in the estimation of renal

³⁰Chace and Myers: Jour. Am. Med. Assn., 1916, vol. lxxvii, No. 13, p. 929.

³¹Addis and Watanabe: Jour. Biol. Chem., 1916, vol. xxiv, p. 203.

function has more recently been taken up by Jonas and Austin.³² They call attention to the fact that in addition to the observations of Addis and Watanabe,³³ Pepper and Austin,³⁴ in dogs, using, however, total nitrogen instead of urea, found enormous variations in the quotient in different animals and in the same animal under different conditions. These two investigators studied the Ambard coefficient as modified by McLean on a number of individuals with presumably normal kidneys and showed that the quotient is anything but constant. In this study which was made on patients in the medical ward of the University of Pennsylvania Hospital, periods of 72 minutes were employed (or in a few instances slightly larger periods up to 160 minutes), and the blood withdrawn from the arm 36 minutes after the period began. The urea was determined by the urease method of Van Slyke and Cullen.³⁵ Their cases were divided into three groups: first, cases in which there was no clinical or laboratory evidence of nephritis, nor of marked cardiovascular disease, nor of cardiac decompensation; second, cases with definite evidence of more or less severe nephritis; third, a few cases in which there was no definite nephritis, but in which there was more or less vascular disease or cardiac decompensation or both. In the first group, there was a wide variation of the index in the same individual on different occasions and in different individuals. The conclusions of these observers on both normal and abnormal cases were:

1. The Ambard formula in its original form or as modified by McLean does not express precisely the law of renal function with respect to the elimination of urea, and this is particularly true as regards the effect of urinary urea concentration.

2. The upper limit of blood urea in nonnephritic and normal individuals under ordinary conditions of diet and life is about 0.35 gm. urea per liter of blood. Figures higher than this are, under ordinary conditions of diet, to be considered evidence of impaired renal function.

3. Using McLean's modification of Ambard's formula, it was found that in the great majority of nephritic cases a lowering of the index was accompanied by an elevation of the blood urea

³²Jonas and Austin: *Am. Jour. Med. Sc.*, October, 1916, vol. clii, No. 4, p. 560.

³³Addis and Watanabe: *Jour. Biol. Chem.*, 1916, vol. xxiv, p. 203.

³⁴Pepper and Austin: *Jour. Biol. Chem.*, 1915, vol. xxii, p. 81.

³⁵Van Slyke and Cullen: *Jour. Biol. Chem.*, 1914, vol. xix, p. 211.

above normal limits, 0.35 gm. per liter, and that the index afforded no information of diagnostic or prognostic value that could not be as readily deduced from the blood urea alone.

4. In certain cases, the index was found to be lowered when the blood urea was within normal limits. This was especially true in arteriosclerotic cases and in cases with cardiac decompensation, which probably detracts from the clinical value of the index as compared with that of the blood urea rather than the reverse, since it is of importance to distinguish between cases of vascular and renal character.

5. In the determination of the index there is a possibility of error arising from undetected incomplete collection of the urine, which cannot occur in the simple blood urea estimation.

6. The urea index estimated repeatedly in the same individual exhibits wider variations in the normal or nonnephritic individual than in the nephritic.

7. For purposes of ordinary clinical diagnosis and prognosis the estimation of blood urea is a more reliable and more useful guide than is the urea index or the Ambard quotient.

Blood Sugar and Nephritis.

Attention must be called to the fact that diabetes may often be complicated by nephritis and that, therefore, the study of blood chemistry of these individuals is most imperative. The presence of undue sugar in the blood and urine of these cases calls attention to the estimation of all the other blood ingredients commonly searched for in nephritis. It must also be remembered that hyperglycemia exists in severe nephritis; this has been recognized for some time by Bang,³⁶ Neubauer,³⁷ Rolly and Oppermann,³⁸ and Hopkins.³⁹ Myers and Bailey⁴⁰ allude to it in connection with an observation of a number of hospital cases. So we may have hyperglycemia with nephritis and nephritis complicating diabetes. Severe nephritis seems to reduce the permeability of the kidney for sugar. In one of their fatal cases, Myers and Bailey point to the marked nephritic symptoms, coupled with a

³⁶Bang: *Der Blutzucker*, Wiesbaden, 1913, p. 128.

³⁷Neubauer: *Biochem. Ztschr.*, 1910, vol. xxv, p. 284.

³⁸Rolly and Oppermann: *Biochem. Ztschr.*, 1913, vol. xlviii, p. 268.

³⁹Hopkins: *Am. Jour. Med. Sc.*, 1915, vol. cxlix, p. 254.

⁴⁰Myers and Bailey: *Jour. Biol. Chem.*, 1916, vol. xxiv, No. 2, p. 147.

high creatinine value of 4.7, indicating that the nephritis had as much to do with the cause of death as the diabetes. In the three fatal cases of diabetes which they studied, the first two showed a normal creatinine value, with an obscure cause of death in both, scarcely acidosis in their opinion. Myers and Bailey reported in this paper a number of cases of nephritis with as high a blood sugar content as 0.20 per cent. In four cases of interstitial nephritis glycosuria was absent, while mild glycosuria was present in the two cases of parenchymatous nephritis with edema. Many of their cases gave evidence of nephritis complicating diabetes. Mosenthal⁴¹ has recently emphasized the fact that cases of interstitial nephritis secrete a urine of a very constant low specific gravity with low content of chloride and nitrogen. It is possible that this same factor may have some influence on the concentration of urinary sugar. Myers and Bailey report one case of 1.10 per cent of blood sugar, possibly the highest figure on record, and only 0.5 per cent in the urine. They state that if the nephritis is of the interstitial type, the data obtained for uncomplicated nephritis explain the elevation of the threshold point of sugar excretion in these advanced cases of diabetes. The nephritis may further explain the difficulty in reducing the blood sugar of these cases to normal by restrictions in the carbohydrate intake. The use of lactose as a functional kidney test has shown quickly the permeability of the kidney for this sugar in nephritis. As an index of the ability of the kidney to excrete sugar, it seems possible that the ratio between the sugar of the blood and urine might be worked out somewhat after the method of McLean,⁴² as recently employed for urea and chlorides.

Blood Chemistry and Surgery.

Operative risk is largely judged by kidney function. Operative risk means ability to stand the anesthetic and to carry on the functions in the presence of an overwhelming change in the organism caused by the operative attack. The methods usually in vogue in surgical institutions to judge kidney function are the routine urinary analysis and the use of the phenolsulphonphthalein test for kidney efficiency. From what has gone before, it seems ra-

⁴¹Mosenthal: Arch. Int. Med., 1915, vol. xvi, p. 733.

⁴²McLean: Jour. Exper. Med., 1915, vol. xxii, pp. 212, 366.

tional to include in this survey of the patient a very complete blood chemical analysis. Since the data already obtained by blood chemical methods have so often upset and changed medical diagnoses and prognoses, it goes without saying that the same set of conditions will occur when these tests are used in connection with surgical procedures. Certainly the surgeon who proceeds to operate after having been assured that the blood sugar, urea nitrogen, uric acid, and creatinine of his patient are within normal bounds, will have far less cause for fear of unforeseen catastrophe to his patients than those who rely simply on the tests commonly used with respect to the urine. Possibly in no department of surgery are these tests so much indicated as in urology in connection with operative procedures upon the old men-candidates for prostatectomy. Remarkable lowering of the death rate from this operation has occurred since the institution of rational preparation of these bad risks for surgery have been carried out, with free washing of the kidney for days prior to the operation by copious drinking of water, the use of diuretics, the awaiting until cardiac and renal functions are within rational limits of health. These patients are examined by the routine methods of urine analysis, special attention being paid to the output of urea without much attention to the blood findings. Estimation of urea without blood urea determinations are necessarily of but little scientific benefit. These tests should be supplemented by urea blood estimations as well as blood sugar and uric acid and creatinine tests.

Aside from the preliminary survey of these operative patients, the surgeon may well utilize the methods of blood chemistry for determination of the impending onset of acidosis in his patients after operation. We hear much of the term acidosis in the surgical hospital, but hear but little of its exact diagnosis. Certain it is, much that is called acidosis in the way of a surgical operation is not acidosis at all and perhaps cases of acidosis occur that are never recognized. It is here that blood chemistry must come forward to settle this question. A rapid estimation of the combining power of the patient's blood plasma by the Van Slyke or Marriott method will speedily clear the picture so far as acidosis is concerned.

A recent study on intestinal obstruction in relation to the non-coagulable nitrogen of the blood is quite interesting along the lines just noted. Cooke, Rodenbaugh, and Whipple⁴³ take up the question of the analytical consideration of blood in cases of intestinal obstruction, intestinal closed loops, and other acute intoxications. Their interest in this question was aroused by a communication of Tileston and Comfort,⁴⁴ who, in a large series of human cases, reported three cases of intestinal obstruction with very high noncoagulable nitrogen. The present writers, Cooke, Rodenbaugh, and Whipple, found that most cases of intestinal obstruction, especially with signs of acute intoxication, showed a high non-coagulable blood nitrogen, and it seemed possible to them that this factor might be of value in diagnosis and especially prognosis of acute abdominal conditions. They have become convinced as a result of their work that this determination of nitrogen in blood is of value in various acute intoxications. If the reading is high, it may be assumed that there exists a dangerous grade of intoxication, but on the contrary, one may not assume that a low reading gives evidence of slight intoxication, because a fatal outcome may be associated with a low reading. It is therefore of considerable value to know that the noncoagulable nitrogen of the blood may show high readings in other conditions than renal disease. On the other hand, determinations of the blood urea alone are of somewhat less value in studying the retention products in the blood in these conditions.

In these animal experiments Cooke, Rodenbaugh, and Whipple found that the blood urea varied less than 30 per cent to more than 80 per cent of the total noncoagulable nitrogen, and while a high urea reading was the rule, the variations in the urea curve and the curves of the other noncoagulable nitrogenous substances were so great that the urea reading was a somewhat unreliable index of the extent to which noncoagulable nitrogenous substances were retained. In these experiments dogs were used mainly, a few cats and one human case being recorded. The blood was taken from the jugular vein in some cases, from the carotid in others. The dogs were anesthetized and loops of the intestine tied off, the

⁴³Cooke, Rodenbaugh and Whipple: *Jour. Exper. Med.*, June, 1916, vol. xxiii, No. 6, p. 717.

⁴⁴Tileston and Comfort: *Arch. Int. Med.*, 1914, vol. xiv, p. 620.

animals watched, blood samples taken at various intervals; in some cases the dogs were reoperated, in other cases they were allowed to die of their intoxications due to obstruction operations. Besides the animal experimental observations, they record one human case of intestinal obstruction, with blood findings.

These experiments showed definite increase in the noncoagulable nitrogen in the blood of cases of intestinal obstruction with closed loops of intestine. With acute intoxication, the rise is shown as striking and constant. This rise was high and was considered a grave sign and was a clinical index of a severe intoxication even in spite of the clinical evidence to the contrary. But a low noncoagulable nitrogen does not guarantee a mild grade of intoxication. Acute proteose intoxication in animals due to the injection of a pure proteose will show a prompt rise in blood noncoagulable nitrogen, even an increase of 100 per cent within three or four hours. These intoxications also showed a high creatinine and urea concentration. The residual or undetermined nitrogen was also high. The human case with autopsy showed the same conditions as the animals under experiment. Clinically the noncoagulable nitrogen of the blood may give information of value in intestinal obstruction. A high reading indicates a grave condition, but a low one may still fail to show a grave intoxication. The kidneys in all these cases at autopsy appeared normal. It is possible that protein or tissue destruction rather than impaired eliminative function was responsible for the rise in noncoagulable nitrogen of the blood in these acute intoxications. Transfusions of dextrose solutions often benefit intestinal obstruction and may depress the level of the noncoagulable nitrogen in the blood. These observers likewise state that some cases show no change in the noncoagulable nitrogen following transfusions and diuresis, and, as a rule, such cases presented the most severe intoxication.

Thus, another line of investigation was opened up by this blood chemical study on intestinal obstruction. Perhaps by this kind of research, the presence of a severe and dangerous grade of surgical complication may be detected before acute clinical symptoms assert themselves.

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